ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTE OF TECHNOLOGY (AAiT)

SCHOOL OF CHEMICAL AND BIO- ENGINEERING

Optimization of Process Parameters for the Extraction of Oil from Mango Seed Kernel (*Mangifera indica*) for Cosmetics Application

A Thesis submitted to Addis Ababa Institute of Technology, In Partial Fulfillment of the requirement of degree of Master of Science in chemical Engineering (Process Engineering Stream)

By: Mustefa Kemal

Advisor: Dr. Eng. Abubeker Yimam

Addis Ababa, Ethiopia

June 2015

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Acronyms

AG: Annex G

AOAC: Association of official analytical chemist

ANOVA: Analysis of variance

AV: Acid value

BHA: Butylated hydroxyanisole

BHT: Butylated hydroxytoluene

CSA: Central statistics agency

FAOSTAT: Food and agricultural organization of statistics division of united nation

FDA: Food and drug administration

FFA: Free fatty acid

ICMD: International chemicals marketing and distribution

LDL: Low density lipoprotein

MKO: Mango kernel oil

MOARD: Ministry of Agriculture and rural development

mm: millimeter

N: Normality

PLC: Private limited company

ROS: Reactive oxygen species

SFE: Supercritical fluid extraction

SG: Specific gravity

SNNPRS: South Nations Nationalities and Peoples Regional State

SXE: Soxhlet extractor

TPC: Total phenolic content

UV: Ultra violet

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Abstract

The main aim of the study was to determine the optimum operating condition for the extraction of oil from mango seed kernel. For this, particle size range of 0.25 mm - 0.5 mm, 0.5 mm - 1.5 mm and 1.5 mm – 3 mm, solvent type hexane, ethanol and petroleum ether and extraction time of 2 hr, 4 hr and 6 hr were considered for optimization. A general factorial design was applied to investigate the effect of process variables on oil yield. Maximum oil yield of 84.81% was obtained for particle size range of 0.25 mm - 0.5 mm at extraction time of 6 hr and with solvent type hexane followed by a yield of 83.33% at particle size range 0.25 mm - 0.5 mm, solvent type petroleum ether and 6 hour extraction time. And minimum oil yield of 18.88% was obtained for particle size range 1.5 mm – 3 mm at extraction time of 2 hr and with solvent type ethanol. ANOVA analysis was showed significant effect of particle size, solvent type and extraction time on oil yield (P value < 0.05). When the particle size range decreased from 1.5 mm – 3 mm to 0.25 mm - 0.5 mm the oil yield increased from 64.07% to 84.81% for solvent type hexane and for 6 hr extraction time. As extraction time was increased from 2 hr to 6 hr the oil yield increased from 57.41% to 84.81% for solvent type hexane and particle size range 0.25 mm - 0.5 mm. Quadratic regression model equations were obtained to describe the effect of variables on the oil yield. The physicochemical properties (total phenolic content, unsaponifiable matter, iodine value, acid value, saponification value, moisture and volatile matter, pH, viscosity, refractive index and specific gravity) of the oil extracted under optimum condition were determined and compared to those of the commercial available mango kernel oil and Indian standards for mango kernel fat. It was found that the physicochemical properties are within the standard except the acid value which is slightly higher. The result also indicated that there is a presence of high unsaponefiable matter (3.85%) and phenolic content (115mg/g for ethanol extract, 83.2mg/g hexane extract and 79.6mg/g for petroleum extract) in the mango kernel oil (obtained from local variety) which makes the oil suitable for cosmetics application.

Key words: Mango seed kernel oil, Total Phenolic content, Unsaponefiable matter, Extraction

Chapter one

Introduction

1.1 Background

Food and Drug Administration (FDA) of United States, which regulates cosmetics, defines cosmetics as "intended to be applied to the human body for cleansing, beautifying, promoting attractiveness, or altering the appearance without affecting the body's structure or functions." Products such as skin creams, lotions, shampoos hair oils, perfumes, lipsticks, fingernail polishes, eye and facial make-up preparations, permanent waves, hair colors, toothpastes, deodorants, and any material intended for use as a component of a cosmetic product are included in this definition (Lawis, 2011).

The total value of the cosmetics market in Ethiopia reached $34 million in 2013 with the average growth rate over the last three years at 19%. Imported products account for around 84% of the market, with the remaining 16% sourced from domestic manufacturers (CSA, 2014). This figure shows that there is a shortage of production of cosmetics locally, one of the major challenges for this is the unavailability of raw material for the production.

In many cosmetic products, oil components form an important part of the formulation. Vegetable oils are rich source of fatty acids and have been successfully used in cosmetic products. Because of their oiling, softening, smoothing and protective properties they are classified to the group of emollients. They make the skin look smooth and properly moistened (Aleksandra and Lzabela, 2014). These oils can be derived from variety of plants and plant parts. Olive oil, corn oil, avocado oil, safflower oil, castor oil, cocoa butter and others are some of the examples (Aleksandra and Lzabela, 2014).

Mango seed kernel oil has been used in the cosmetics industry as ingredient in soap, shampoos and lotions because, in addition to having an application as vegetable oil, it is a good source of phenolic compounds (Soong and barlow, 2004) including microelements like selenium, copper and zinc (Schiber *et al*., 2003). In addition, the extract of mango seed kernel exhibited the highest degree of free-radical scavenging and tyrosinase-inhibition activities compared with methyl gallate and phenolic compounds from the mango seed kernel and methyl gallate in emulsion affected the stability of the cosmetic emulsion systems (Kittiphoom and Sutasinee, 2013). Presence of high unsaponifiable matter guarantees the use of mango kernel oil in

cosmetics industry (Nzikou et al., 2010; Saiprabha et al., 2011). It ranges from 1.0 to 5.3% (lakshminarayana et al., 2008)

Mango kernel oil also called mango butter reduces degeneration of skin cells and restores elasticity. Some dermatologists recommend mango butter for treatment of wrinkles. It has a protective effect against harmful UV radiations from sun. Mango seed kernel oil and its derivatives are used in cosmetic as a preservative since it has high content of stearic acid (38%). It melts at body temperature or upon contact with skin and disperses smoothly, providing a protective, emollient layer (Saiprabha and Goswami-Giri, 2011; Nzikou *et al.*, 2010). So using mango seed oil as adding chemical in cosmetics industry can comprise both economical and environmental benefit because it is a waste.

Mango is one of the second potential fruit crop produced in Ethiopia next to banana which is the first fruit crop produced in large quantity and produced mainly in-west and east of Oromia, SNNPR, Benishangul (Assosa) and Amhara regional states (Takele, 2014). More than 47 thousand hectares of land is under fruit crops in Ethiopia. Mangoes contributed about 12.61% of the area allocated for fruit production and took up 12.78% of fruit production in comparison to other fruits growing in the country (Elias, 2007). Production of mango in 2012/2013 cropping season at SNPP is 343,910.27 quintal and a total area coverage is 3,375.89 hectare and that of Assosa and oromia are 51,411.10 quintal and a total area coverage of 652.56 hectare and 284,065.27 quintal and total area coverage of 3,789.47 hectare respectively (CSA, 2013). This yield is certainly impressive and conditions are very well suited for mango production in Ethiopia.

In Ethiopia there are many large and small scale mango juice processing industry. During processing of mango, by-products such as peel and kernel are generated and kernels take up about 17-22% of the fruit. Even if the kernels have important application in cosmetics industry currently it is not utilized for any commercial purposes, it is discarded as a waste and becomes a source of pollution. So production of oil from the mango seed kernel supports cosmetics industry in the production of different cosmetics materials and also minimizes cost. It is intended that this thesis will be useful in providing the necessary process design information needed for the production of oil from mango seed kernel.

1.2 Statement of the problem

Due to high population growth, finding an alternative source for producing valuable products is becoming crucial thing at present. A more convenient way is to use by- products (wastes) as a potential sources due not only reduce the cost of production but also environmental and health effect of wastes.

In Ethiopia there are many small scale (juice houses) and large scale juice processing industries, one of the by-product of this industry is mango seed. It is estimated that mango processing yields about 40 – 50% of by-product (waste) which may cause environmental problem (Sruamsiri et al., 2009 ; De la Cruz 2005).

Different studies showed that mango seed kernel (one of the by-product of juice processing industries) have 8- 16% oil content which is an excellent source of phenolic compounds which are responsible for antioxidant activity, skin whitening and anti-wrinkle agents (Soong et al., 2004; González et al., 2008). And also reach in saturated fatty acids mostly stearic and palmatic acid and unsaturated fatty acids mostly oleic and linioleic acid which contribute several beneficial properties in cosmetics and personal care products (Vermaak et al., 2011). So the oil is essential in cosmetics industry. However, in Ethiopia peoples ignorantly throw away the seeds after using the fruit part and it becomes a source of pollution. This is may be due to a limited knowledge of toxicological status of the kernel functional property and appropriate process technology.

In Ethiopia there are many cosmetics industries e.g. Berchaco Ethiopia pvt.Ltd, Zenith Gebs Eshet ETH.Ltd and also other cosmetics manufacturing industry which produces different hair and body care cosmetics material. This industry imports almost all of the raw material for the production, so producing oil from local mango kernel waste minimizes the production cost and also producing in large scale will be source of income.

Different literatures reported that process parameters such as particle size, extraction time, solvent type, solid to solvent ratio, temperature and moisture content have a significant effect on oil yield. Particle size, extraction time and solvent type are selected for this study based on results from previous work. Thus, this thesis aims to find the optimum operating conditions for the production of oil from mango seed kernel that will maximize the oil yield.

1.3 Objectives

1.3.1 General objective

The general objective of the study was to optimize extraction process parameters for maximum yield of oil from mango seed kernel using full factorial design.

1.3.2 Specific objectives

The specific objectives were the Following:

- To characterize the extracted oil.
- To investigate the effect of particle size, solvent type and extraction time on quantity of oil (oil yield).
- To compare the physicochemical property of the oil extracted at optimum operating condition with that of commercially available mango kernel oil used for cosmetics application.

1.4 Significance of the study

The significances of the production of oil from mango seed kernel are,

- The oil can be used as an ingredient in producing different types of mango kernel oil based skin and hair caring cosmetics materials so, different cosmetics industry will be benefited and also substitutes import.
- Production of oil from mango seed kernel will contribute for minimizing environmental pollution, since mango seed is one of the wastes of juice processing industries. And also generates an income for juice processing industries.
- Mango kernel oil production program can also be helpful to creates job opportunity for the society.
- The result of this study will be used as a base line information for future study since the oil has also an application in pharmaceutical industry and also used as edible oil.

Chapter Two

Literature review

2.1 Historical Background of Mango

The mango is a very common tropical fruit usually found in Southern Asia, especially in Eastern India, China, Burma, Andaman Islands and Central America (Kittiphoom, 2012). It is cultivated and grown vastly in many tropical regions and widely distributed in the world. Mango is indigenous to the Indian subcontinent and Southeast Asia (Fowomola, 2010). It is one of the most extensively exploited fruits for food, juice, flavor, fragrance and color and a common ingredient in new functional foods often called superfruits (Kittiphoom, 2012). Due to its attractive color, delicious taste and exotic flavor it has been recognized as 'king of the fruit' (Pott et al.,2003).

Mangos belong to the genus *Mangifera* of the family Anacardiaceae. The genus *Mangifera* contains several species that bear edible fruit. Most of the fruit trees that are commonly known as mangos belong to the species *Mangifera indica* (Singh, 1996). Mango (*Mangifera indica*) is one of the most important tropical fruits in the world and currently ranked 5th in total world production among the major fruit crops (FAO, 2004).

2.2 Description of Mango tree

Mango trees (*Mangifera indica*) reach 35 - 40 m in height, with a crown radius of 10 m. The leaves are evergreen, alternate, simple, 15 - 35 cm long and 6 - 16 cm broad; when the leaves are young they are orange-pink, rapidly changing to a dark glossy red, and then dark green as they mature. The fruit takes from 3 - 6 months to ripen (Fowomala, 2010). The mango fruit is a large fleshy drupe, highly variable in size, shape, color and taste and weighing up to 1 kg in some cultivars. Green when unripe, the fruit turns orange-reddish as it ripens. The fruit consists of a woody endocarp (pit), a resinous edible mesocarp (flesh) and a thick exocarp (peel) (Heuzev et al., 2005).

Mango seed is a single flat oblong seed that can be fibrous or hairy on the surface, depending on the cultivar. Inside the seed coat 1 - 2 mm thick is a thin lining covering a single embryo, 4 - 7 cm long, 3 - 4 cm wide, and 1 cm thick. Mango seed consists of a tenacious coat enclosing the kernel. The seed content of different varieties of mangoes ranges from 9% to 23% of the fruit weight and the kernel content of the seed ranges from 45.7% to 72.8% (Khammuang et al., 2011)

2.3 Geographical description

2.3.1 Overview of mango production in the world

Mangoes are produced in over 90 countries worldwide. Asia accounts for approximately 77% of Global mango production, and the Americas and Africa account for approximately 13% and 9%, respectively (FAOSTAT, 2013).

In 2012, world production of mango was estimated at 36.1 million metric tons. Between 2008 and 2012, production grew at an average annual rate of 2.6%. Table 2.1 shows the world's top ten mango producing countries, which account for about 90% of the world's mango production. (FAOSTAT, 2014)

Table 2.1 World's top ten mango producers, 2008 – 2012

countries	2008	2009	2010	2011	2012	2011-2012
India	466,436	12,750,000	15,026,700	15,188,000	15,250,000	46.2
China	3,976,716	4,140,290	1,287,287	2,131,139	4,567,247	10.2
Thailand	2,374,165	2,469,814	2,550,595	2,600,000	2,650,000	8.0
Pakistan	1,753,686	1,727,932	1784	1,845,528	1,950,000	5.8
Mexico	1,716,537	1,509,272	1,632,649	1,827,314	1,760,588	5.4
Indonesia	2,105,085	2,243,440	1,287,287	2,131,139	2,376,339	6.8
Brazil	1,154,649	1,197,694	1,189,651	2,131,139	1,175,735	5.0
Philippines	884,011	785,510	843,508	800,551	783,225	2.4
Egypt	466,436	534,434	505,741	505,741	2,781,706	5.0
Kenya	448631	528815	593499	636585	2781706	5.2

(FAOSTAT, 2014)

India is the largest producer of mangoes, accounting for 46.2% of world production from 2011 to 2012. During that period, India's mango crop averaged 30.44 million metric tons, followed by China and Thailand at 6.70 million metric tons (10.2%) and 5.25 million metric tons (8.0%), respectfully. Other leading mango producers during the 2011 to 2012 period include Pakistan (5.8%), Mexico (5.4%), Indonesia (6.8%), Brazil (5.0%), Philippines (2.4%), Egypt (5.0%) and Kenya (5.2%).

2.3.2 Overview of mango production in Ethiopia

Ethiopia is agro-ecologically diverse and has a total area of 1.13 million km^2. Many parts of the country are suitable for growing temperate, sub-tropical or tropical fruits. For example, substantial areas in the southern and south-western parts of the country receive sufficient rainfall to support fruits adapted to the respective climatic conditions (Amer, 2002). There are also regions within Ethiopia that are well suited to producing a surplus for particular agricultural commodities. One such location is the Asossa – Homosha region in western Ethiopia, which is particularly suitable to the production of mangoes (James et al., 2009).

The Ethiopian government has a plan to expand mango production by distributing high yielding varieties for small scale farmers, especially in the Southern and Oromia region, by grafting mangos of known and high yielding varieties. In July 2006, it was announced that the Oromia Government distributed 14,000 improved seeds of mango. The production of mango fruits for the past Nine years in Ethiopia according to FAOSTAT (2014) is shown in table 2.2

Table 2.2 Estimate of area, production and yield of mango fruit Meher season, Ethiopia

Year	**Area in hectare**	**Production in quintal**	**Yield Qt/hectare**
2003/04	4,964.00	292,283.00	58.88
2004/05	5,814.00	301,715.00	51.89
2005/06	5,400.31	547,291.24	104.06
2006/07	6,796.10	626,111.83	94.08
2007/08	6,730.83	484,360.97	71.96
2008/09	6,051.00	441,582.06	72.97
2009/10	8,630.00	656,200.00	76.04
2010/11	7,513.00	656,520.00	87.38
2012/13	8,809.00	697,510.00	79.18

(FAOSTAT, 2014)

2.3.3 Mango Market in Ethiopia

It is common in Ethiopia that majority of mango producers sell their products at nearby local market. Most of the time mango producers sell their product to consumers and sometimes to retailers because of the market fluctuation and lack of marketing infrastructures and also since, maturity stage and harvesting time of mango fruit is similar, this condition increase the supply at that time and the demand is less compare to that of the supply. In this situation the price of the fruit become less and less and as result of this farmers are obliged to sale their produce at local market. In addition to this, farm gate sale of mango is also common in Ethiopia. The main sales channels of mango in Ethiopia include direct sale to consumer, hotels, large retailers and supermarkets, wholesalers and small retailers and kiosks (Takele, 2014).

The local market is one of potential option for growers to sell their fruit. Most of the top quality fruit is collected and transported by the traders destined for Addis Ababa market, and the remainder is sold at the local level. The Addis market is dominated by two large wholesale markets, being the Mercato and the Piazza. These markets are the main destination for agricultural produce arriving from around the country. These markets serve not only consumers, but are also where supermarkets, large retailers, hotels and thousands of small kiosk-like retailers (James et al., 2008). Wholesale Mango Market in Addis Ababa is dominated by Assosa mango (28%) as shown in the figure below. So, for this study assosa mango seed kernel was considered due to its high market share and due to its preferebility by most people due to it high quality and its relative brand strength compared with mangoes from other regions.

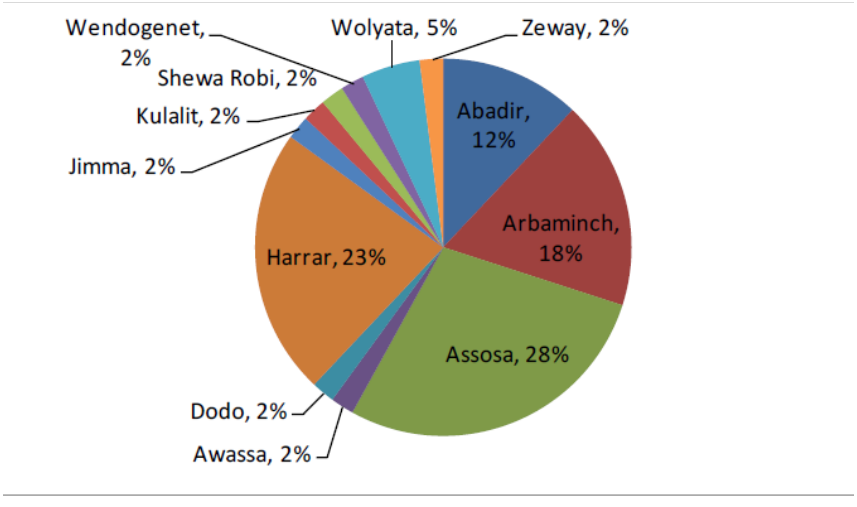

Figure 2.1 Wholesale Mango Market in Addis Ababa – Market share by the regions

2.4 Mango variety

Mango is generally considered to be one of the most important fruits of tropical regions. In most tropical regions fruit is grown only for local consumption but it is produced for export in India, Hawaii, Mexico, Brazil and parts of Africa. In Africa, mango has become naturalised due to germinating discarded seeds and grows wild in most inhabited areas.

There are hundreds of varieties mango world-wide. They vary in colour, shape and flavour. Some are eaten green (Alstonvill, 2004).

Most common varieties of Mango available in Ethiopia:

Kent

The tree is vigorous, with a compact growth habit. It produce high yield and large fruits, with oval shape. The palatability is excellent, with rich sweet flavour. The skin colour is greenish-yellow with some rer blush when ripe. The flesh is fibreless.

The seed is monoembryonic (Giueseppe De Bac, 2010).

Figure 2.2 Kent variety mango

Keitt

The tree is also vigorous but a bit smaller than Kent. Fruit production is large and consistent. The fruit shape is oval with a slight beak at the bottom which differentiates its shape.

Skin colour is green with some light red blush. The flesh is fibreless with monoembryonic Seed and the fruit has a good disease resistance (Giueseppe De Bac, 2010).

-+ 

Figure 2.3 keitt varaity mango

Tommy Atkins

Very popular for the trade, thanks to its longer shelf life. The tree habit is compact. The yield is high. Fruit is oval compact with characteristic stunning purplish colour. The flesh is fibreless, with no very high sugar content and sweet flavor (Giueseppe De Bac, 2010).

Figure 2.4 Tommy Atkins variety mango

Apple Mango

Originally from the Far East. The tree is compact, the yield quite good, the fruit has an attractive flavour, fibreless flesh, small seed and round shape from which it is named Although there are many ways of propagating mango by grafting , cleft grafting is the most successful and popular under local conditions (Giueseppe De Bac, 2010).

Figure 2.5 Apple mango variety

2.5 Some health benefits of mango fruit and mango seed

Mango fruit is rich in pre-biotic dietary fiber, vitamins, minerals and poly-phenolic flavonoid antioxidant compounds. It is rich in amino acids, vitamins C, A and E, flavonoids, beta-carotene, niacin, calcium, iron, magnesium and potassium.

According to new research study, mango fruit has been found to protect against colon, breast, leukemia and prostate cancers. Several trial studies suggest that polyphenolic anti-oxidant compounds in mango are known to offer protection against breast and colon cancers.

Mangoes contain digestive enzymes that help break down proteins, aiding digestion. It is also helpful against heartburn due to an enzyme present in it, which soothes the stomach. Due to the large amount of fiber in the handle, may be useful for preventing constipation.

Mango is effective in relieving clogged pores of the skin. This means that people who suffer from acne will benefit from the handle. Simply remove the mango pulp and apply on the skin for about 10 minutes before washing. Eating handle makes your skin whiter, smoother and shinier (Dr. Gustavo Gonalez et al., 2009).

Mango seed is a single flat oblong seed that can be fibrous or hairy on the surface. The seed consists of a tenacious coat enclosing the kernel. The seed content of different varieties of mangoes ranges from 9% to 23% of the fruit weight and the kernel content of the seed ranges from 45.7% to 72.8% (Palaniswamy et al., 1974).

Mango seed kernel contain crude protein, oil, ash, crude fiber, and carbohydrate.

Table 2.3 Proximate composition of mango seed kernel

Characteristics	Values (M±S.D)
Moisture content (%)	45.2±0.17
Crude protein (%)	6.36±1.07
Fat /Oil	13.0±1.28
Crude fiber (%)	2.02±0.80
Ash content (%)	3.2±0.30
Total carbohydrate (%)	32.24

(Nzikou, et al., 2010)

Mango seed kernel is high in potassium, magnesium, phosphorus, calcium and sodium. Potassium is an essential nutrient and has an important role is the synthesis of amino acids and proteins (Malik and Srivastava, 1982). Calcium and magnesium plays a significant role in photosynthesis, carbohydrate metabolism, nucleic acids and binding agents of cell walls (Scalbert, 1991). Calcium assists in teeth development (Brody, 1994). Magnesium is essential mineral for enzyme activity, like calcium and chloride; magnesium also plays a role in regulating the acid-alkaline balance in the body. Phosphorus is needed for bone growth, kidney function and cell growth. It also plays a role in maintaining the body's acid-alkaline balance (Fallon and Enig, 2001).

Mango seed contains 15.27 (IU) vitamin A; (1.30 mg/100 g) vitamin E; (0.59 mg/100 g) vitamin K; (0.08 mg/100 g) vitamin B1; (0.03 mg/100 g) vitamin B2; (0.19 mg/100 g) vitamin B6; (0.12 mg/100 g) vitamin B12 and (0.56 mg/100 g) vitamin C. These results also showed that mango seed is richer in vitamins.

2.6 Application (Importance) of mango seed oil

Mango Seed oil has a treasure-house of nutritional benefits including antioxidants, fatty acids and other health-supporting components. It is an excellent choice for skin care preparations aiding in hydration, elasticity and sun-protective qualities.

The chemical properties of mango seed oil are amongst the most important properties of the oil . Free fatty acid and peroxide values are always used as an index of oil quality. The low free fatty

acid of mango seed oil indicated that the mango seed was almost free from hydrolytic rancidity brought almost by lipases and enables the direct use of such as oil in industries without further neutralization (Arogba, 1999).

The major saturated fatty acids in mango seed kernels oil were stearic and palmitic acids and the main unsaturated fatty acids are oleic and linoleic acids . The comparison of the composition in fatty acids of mango seed kernel oil with that of vegetable oils indicates that this plant is rich in acids stearic and oleic. Accordingly, mango seed kernel oil is more stable than many other vegetable oils rich in unsaturated fatty acids. Such oils seem to be suitable for blending with vegetable oils, stearin manufacturing, confectionery industry or/and in the soap industry (Kittiphoom, 2012).

Some of the Application of mango seed kernel oil are:

2.6.1 Cosmetics

Mango kernel oil has been used in the cosmetics industry as an ingredient in soaps, shampoos and lotions because it is a good source of phenolic compounds (Soong and Barlow, 2004). The potential use of phenolic compounds for the development of new skin care cosmetics has been emphasized. Phenolic compounds can be used as skin whitening, sunscreen and anti-wrinkle agents (González et al., 2008). Melanin is the root cause for darkening of the skin. Its formation beneath the skin proceeds through a free-radical mechanism. UV-radiations facilitate this chain reaction and it could be disrupted by selective use of compounds, potent enough to inhibit this reaction (Choi et al., 2007). It is well documented that tyrosinase is an essential enzyme, which contributes towards pigment formation in a mammal's body as well as in plants, microorganisms and fungi (Choi et al 2007). The use of tyrosinase inhibitors is becoming increasingly important in the cosmetic industry due to their skin-whitening effects.

The mango seed kernel was shown to be a good source of phenolic compounds (Soong and Barlow, 2004) including microelements like selenium, copper and zinc (Schiber *et al*., 2003). In addition, the extract of mango seed kernel exhibited the highest degree of free-radical scavenging and tyrosinase-inhibition activities compared with methyl gallate and phenolic compounds from the mango seed kernel and methyl gallate in emulsion affected the stability of the cosmetic emulsion systems. It can be concluded that mango seed kernel oil can be use as adding chemicals and ingredients cosmetics and pharmaceuticals (Kittiphoom, 2012).

The main components of unsaponifiables in vegetable oils are tocopherols (vitamin E) and sterols, present in different amounts. Tocopherols are recognized as very efficient natural antioxidants and their amount in the plant is probably governed by the content of unsaturated fatty acids. Tocopherols are about 250 times more effective than butylated hydroxytoluene (BHT) (Burton and Ingold, 1989).

The presence of high unsaponifeble matter in vegetable oil can grant the use of the oil in cosmetics industry. Since mango kernel oil has relatively high unsaponifeble matter it is highly garanted to be used in cosmetics industry (Nzikou et al., 2010; Saiprabha et al., 2011). The unsaponefiable matter in mango seed kernel oil ranges from 1.0 to 5.3% (lakshminarayana, 2008 et al).

Mango oil also called mango butter reduces degeneration of skin cells and restores elasticity. Some dermatologists recommend mango butter for treatment of wrinkles. It has a protective effect against harmful UV radiations from sun. Mango seed kernel oil and its derivatives are used in cosmetic as a preservative since it has high content of stearic acid (38%). It melts at body temperature or upon contact with skin and disperses smoothly, providing a protective, emollient layer (Saiprabha and Goswami-Giri, 2011; Nzikou *et al.*, 2010).

2.6.2 Mango seed oil as alternative to the high value product cocoa butter

Cocoa butter is the fat extracted from the Theobroma cacao seeds (Becktt, 2000) that is commonly used as an ingredient in several confectionery products, especially in chocolate due to its specific properties. Cocoa butter is one of the most expensive vegetable oils consisting mainly of palmitic acid, stearic acid and oleic acid and a trace amount of lauric acid and myristic acid (Kheiri, 1982; Pease, 1985). So industries have tried to look for alternative vegetable oils that have chemical and physical properties similar to cocoa butter but they are cheaper. The research from Kaphueakngam et al. (2009) found that oil obtained from the mango seed kernels could be an alternative source of edible oil. With the right proportion, an oil blend between mango seed kernel oil and palm oil could be used as Cocoa butter. Fatty acid compositions of mango seed kernel oil, mixture of mango seed kernel oil/palm oil and cocoa butter are given in Table 2.4. The results show that, like cocoa butter, the mixture of mango seed kernel oil/palm oil was composed mainly of palmitic acid, stearic acid and oleic acid. Hence, mango seed kernel oil is a

good source of stearic acid and oleic acid whereas palm oil is a source of palmitic acid and oleic acid. The 80/20 (%wt of mango seed kernel oil/palm oil) blend mainly consisted of three fatty acids that were also the main fatty acid components of cocoa butter, the melting behavior and slip melting point were closest to that of cocoa butter (Kaphueakngam et al).

Table 2.4 Fatty acid composition for cocoa butter mango, seed oil, palm oil and mixture of mango kernel oil and palm oil

Fatty acid	Mango seed kernel oil	Palm oil	Cocoa butter	80/20 (% wt of mango seed kernel oil /palm oil)
Palmitic	9.29	51.65	24.27	16.26
Stearic	39.07	4.21	35.10	37.25
Oleic	40.81	35.63	36.47	36.6
Linoleic	6.06	-	2.85	-
Linolenic	0.64	-	0.30	-
Arachidic	2.48	-	1.01	-
Behenic	0.64	-	-	-
Lignoceric	0.49	-	-	-

(Solis-Fuentes and Duran-de-Bazua, 2004; Kaphueakngam *et al*., 2009)

Cocoa Butter has been used in high quality cosmetics for centuries. More and more, the constituents of cocoa, such as polyphenols, are linked to favourable health effects, in particular related to their anti-inflammatory properties and the antioxidant properties which may help to reduce oxidation of LDL-cholesterol and fight reactive oxygen species. In other words, they can form a factor against atherosclerosis, heart diseases and a large variety of illnesses related to reactive oxygen species. In addition, cocoa has also been linked to a favourable effect on the immune system (vegitables and oils ICMD).

2.6.3 Natural antioxidant

Vegetables and fruits have been used as natural materials to maintain human health as they may help to reduce the risk of many age-related degenerative diseases (Amin and Tan, 2002; John and James, 2005; Lee *et al*., 2007). In fact, fruits contain many antioxidant compounds such as phenolics, betalains and carotenoids. Antioxidants can be defined as substances able to inhibit or delay the oxidative damage of protein, nucleic acid and lipid caused by dramatic increase of reactive oxygen species (ROS) during environmental stress (Lim *et al.*, 2006). Antioxidants act by one or more of the following mechanisms: reducing free radical activity, scavenging free radicals, potential complexing of pro-oxidant metals and quenching of singlet oxygen (Tachakittirungrod *et al*., 2006). Antioxidants can be classified into primary and secondary antioxidants due to their protective properties at different stages of the oxidation process. Primary antioxidants stop or delay oxidation by donating hydrogen atoms or electrons to free radicals to convert themselves to more stable products. As for secondary antioxidants, they function by many mechanisms, including binding of metal ions, scavenging oxygen, converting hydroperoxides to nonradical species, absorbing UV radiation or deactivating singlet oxygen (Maisuthisakul *et al*., 2005).

Food antioxidants are compounds or substances that are present naturally in some ingredients or are intentionally added as food additive with the aim of inhibiting product oxidation (Halliwell, 1996). Thus, their use in the food industry is important to maintain quality, mainly in foods that contain high levels of lipids, such as meat products. In industrial processing, synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), are frequently used in order to inhibit the formation of free radicals and to prevent lipid auto oxidation and food spoilage. Synthetic antioxidants show good stability during processing and storage of high lipid foods. In recent years, however, many countries (Japan and some European countries) have suppressed the use of synthetic antioxidants because of their potential toxicity and carcinogenicity (Wanasundara and Shahidi, 1998; Tang *et al*., 2001). Thus, the demand for antioxidant substances naturally found in fruits and vegetables has increased since the use of those substances in products has been considered due to healthy benefits to consumers such as the reduction in the incidence of cardiovascular diseases and cancer. Moreover, natural antioxidants can be more effective in retarding food oxidation (Zandi and Gondon, 1999; Kang *et al*., 2001; Bub *et al*., 2003). The importance of natural antioxidants for cosmetic and food

applications has been underlined by numerous works. The limitations of using a plant extract as a natural antioxidant are availability and cost. Several studies have shown that mango seed kernels contain various phenolic compounds and can be a good source of natural antioxidants (Puravankara *et al*., 2000; Abdalla *et al*., 2007). In addition, polyphenols from mango seed kernels were found to contain tannins, gallic acid, coumarin, ellagic acid, vanillin, mangiferin, ferulic acid, cinammic acid (Arogba, 1997). The total antioxidant capacity and phenolic content vary considerably from one kind of fruit to another. Antioxidant activity and phenolic content of seeds of avocado, jackfruit, longan, mango and tamarind were shown in Table 9. Mango seed kernel had the highest antioxidant activity and phenolic content, followed by the seeds of tamarind, avocado, longan and jackfruit. This suggests that the fruit seeds should be further utilized rather than just discarded as waste.

Table 2.5 Total phenolic contents and antioxidant activity of different seeds

Item	Phenolic content mg / g	Antioxidant activity μmol / g
Mango kernel	117±13.5	762±72.9
Avocado seed	88.2±2.2	236.1±45.1
Jackfruit seed	27.7±3.4	7.4±2
Tamarind seed	94.5±4.9	698±30.3
Longan seed	62.6±3.2	488±82.5

(Soong and Barlow, 2006)

The main components of unsaponifiables in vegetable oils are tocopherols (vitamin E) and sterols, present in different amounts. Tocopherols are recognized as very efficient natural antioxidants and their amount in the plant is probably governed by the content of unsaturated fatty acids. Tocopherols are about 250 times more effective than butylated hydroxytoluene (BHT) (Burton and Ingold, 1989). The high potency of tocopherols as antioxidants and quenchers of singlet oxygen is based on their ability to be transformed back from the oxidized form into the active structure by other molecules such as ascorbic acid and glutathione (Tapel, 1998).

Mango seed kernel extracts enhanced oxidative stability of fresh-type cheese and ghee and extended their shelf life (Parmar and Sharma, 1990; Puravankara *et al*., 2000; Dinesh *et al*.,

2000). This could be attributed to the phospholipids and phenolic compounds, tocopherols and carotenoids (Kittiphoom, 2012). Youssef (1999) indicated that adding 1% of crude oil extracted from mango seed kernel exhibited antioxidant potency similar to that of 200 ppm of butylated hydroxytoluene (BHT).

It can be concluded that mango seed kernel oil can be used as natural antioxidant in different kind of foods, due to high content of different phenolic compounds, tocopherols, and different sterols. Although the antioxidant properties of natural products have been widely recognized, natural antioxidants are still not widely used due to high costs, color and flavor problems. However, the high amounts of antioxidants in the mango kernels indicate that it may be commercially feasible.

2.6.4 Nutrition

On the nutritional and toxicological studies of the mango seed kernel ,Rakmini *et al*.(1989), indicated that mango seed kernel fat is promising and a safe source of edible oil and was found to be nutritious and non-toxic so that it could be substituted for any solid fat without adverse effects. Rashwan (1990) also showed that the lipids extracted from different mango varieties were free from toxic material such as hydrocyanic acid. The principal fatty acids in mango seed kernel oil are 37.73% stearic acid and 46.22% oleic acid and the proportion of unsaturated fatty acids was greater than the saturated fatty acids (1.3%). Such oils seem to be suitable for blending with vegetable oils (Maryam and Mohammed, 2013).

2.7 Classification of Vegetable oils

Vegetable oils are triglycerides extracted from plants. Such oils have been part of human culture for millennia. Edible vegetable oils are used in food, both in cooking and as supplements. Many oils, edible and otherwise, are burned as fuel, such as in oil lamps and as a substitute for petroleum-based fuels. Some of the many other uses include wood finishing, oil painting, and skin care (Robin Dand, 1999). Vegetable oils can be classified in several ways, for example:

- By source: most, but not all vegetable oils are extracted from the fruits or seeds of plants, and the oils may be classified by grouping oils from similar plants, such as "nut oils".

- By use: oils from plants are used in cooking, for fuel, for cosmetics, for medical purposes, and for other industrial purposes (Economic Research Service 2011).

The vegetable oils are grouped below in common classes of use.

Edible oils

Cooking oil is plant, animal, or synthetic fat used in frying, baking, and other types of cooking. It is also used in food preparation and flavouring that doesn't involve heat, such as salad dressings and bread dips, and in this sense might be more accurately termed edible oil. Edible oil is typically a liquid at room temperature, although some oils that contain saturated fat, such as coconut oil, palm oil and palm kernel oil are solid. Types of edible oil include: olive oil, palm oil, soybean oil, canola oil (rapeseed oil), pumpkin seed oil, corn oil, sunflower oil, safflower oil, peanut oil, grape seed oil, sesame oil, argan oil, rice bran oil and other vegetable oils, as well as animal-based oils like butter and lard (O'Brien, 1998).

Oils used for biofuel

A number of oils are used for biofuel addition to having other uses. Vegetable oils are evaluated for use as a biofuel based on:

1. Suitability as a fuel, based on flash point, energy content, viscosity, combustion products and other factors
2. Cost, based in part on yield, effort required to grow and harvest, and post-harvest processing cost (Lee and Shah, 2012).

Caster oil, mustard oil, palm oil, rice brain oil, peanut oil, soybean oil, corn oil and others are edible oils and also they can be used for biofuel production . Copaiba, Jatropha oil, Jojoba oil, Milk bush, Nahor oil, Paradise oil, Pongamia oil this oils are inedible oil extracted from plant part and primarily used as biofuel (Gunstone, 2009).

Drying oils

Drying oils are vegetable oils that dry to a hard finish at normal room temperature.

Such oils are used as the basis of oil paints, and in other paint and wood finishing applications (Encyclopedia of Painting Materials).Dammar oil, Linseed oil, Poppyseed oil, Tung oil, and Vernonia oil are classified under this class.

Other oils

There are number of vegetable oils are either not edible, or not used as an edible oil like Carrot seed oil, Neem oil, Rubber seed oil, Rubber seed oil. Mango oil pressed from the stones of the mango fruit is classified under this class (Morton, 2014).

2.8 Methods for extraction of oil

The extraction technique used to obtain high aggregate value compounds from natural products is crucial for product quality (Kittiphoom and Sutasinee, 2013). The production process of oil involves the removal of oil from plant components, typically seeds. This can be done via mechanical extraction using an oil mill or chemical extraction using a solvent. The extracted oil can then be purified and, if required, refined or chemically altered. Extraction of oil from mango seed kernel can be performed using three different methods: mechanical extraction, solvent extraction, and supercritical fluid extraction.

2.8.1 Mechanical extraction

Oils can be removed via mechanical extraction, termed "crushing" or "pressing." This method is typically used to produce the more traditional oils (e.g., olive, coconut etc.), and it is preferred by most "health-food" customers in the United States and in Europe. There are several different types of mechanical extraction. Expeller-pressing extraction is common, though the screw press, ram press, and Ghani (powered mortar and pestle) are also used. Oil seed presses are commonly used in developing countries, among people for whom other extraction methods would be prohibitively expensive; the Ghani is primarily used in India (Janet Bachmana).

The mechanical extraction method is effective for seed contain 30-70% oil. This method has several advantages compared to the other methods, such as simple equipment and low investment, low operating cost, and the oil does not undergo solvent separation process. However, the oil produced with this method usually has a low price, since it’s turbid and

contains a significant amount of water and metals contents. Due to low oil content of mango seed kernel it is not advisable to extract the oil using mechanical extraction.

2.8.2 Solvent extraction

Solvent extraction is the transfer of solutes from a solid, usually in particulate form, to contiguous liquid, the extract (Henry, 1983). If the solute is uniformly dispersed in the solid, the material near the surface will be dissolved first, leaving a porous structure in the solid residue. The solvent will then have to penetrate this outer layer before it can reach further solute, and the process will become progressively more difficult and the extraction rate will fall. If the solute forms a very high proportion of the solid, the porous structure may break down almost immediately to give a fine deposit of insoluble residue, and access of solvent to the solute will not be impeded. Generally, the process can be considered in three parts: first the change of phase of the solute as it dissolves in the solvent, secondly its diffusion through the solvent in the pores of the solid to the outside of the particle, and thirdly the transfer of the solute from the solution in contact with the particles to the main bulk of the solution. Any one of these three processes may be responsible for limiting the extraction rate (Richardson et al., 2002).

With seeds such as mango seed containing only about 8 - 16 per cent of oil, solvent extraction is often used because mechanical methods are not very efficient.

The solvent extraction method recovers almost all the oils and leaves behind only 0.5% to 0.7% residual oil in the raw material. In the case of mechanical pressing the residual oil left in the oil cake may be anywhere from 6% to 14%. The solvent extraction method can be applied directly to any low oil content raw materials. Because of the high percentage of recovered oil, solvent extraction has become the most popular method of extraction of oils and fats.

The advantages of solvent extraction over other methods of oil expression include, higher oil yield (about 95% of the oil content could be obtained), larger processing capacity, solvent extraction also gave oil that many considered to be of superior bleaching quality, lower refining losses, reduced susceptibility to rancidity and better retention of fat - soluble vitamin (Lawson et al., 2010).

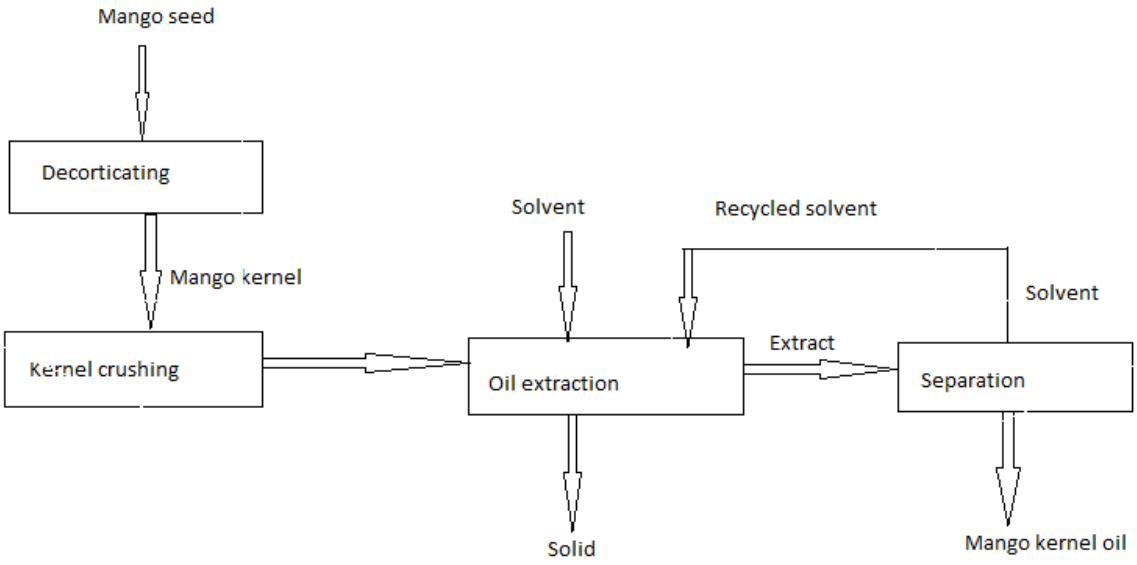

Figure 2.6 Solvent extraction of mango seed

A certain gram of dried mango seed kernel was used in the process. The seeds firstly decorticated to obtain the seed kernels. Then dried and milled to a certain particle size and then fed to an extractor. After waiting to a certain time, the separation process proceeds to separate the solvent from the product. The recycled solvent was used again by adding certain make up solvent.

2.8.2.1 Conventional solvent (solid-liquid) extraction

Conventional solvent extraction methods include maceration, percolation, sonication and Soxhlet extraction (SXE), while newer techniques comprise ultrasoundand microwave-assisted extraction, as well as pressurized liquid extraction (PLE).

Soxhlet Extractor

A Soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. It was originally designed for the extraction of a lipid from a solid material. However, a Soxhlet extractor is not limited to the extraction of lipids. Typically, a Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a significant solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. Mango seed kernel powder is placed inside a thimble made from thick filter paper, which is loaded into the main

chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser .

The solvent is heated to reflux. The solvent vapor travels up a distillation arms and floods into the chamber housing the thimble of mango kernel powder. The condenser ensures that any solvent vapor cools, and drips back down into the chamber housing of the kernel powder.

The chamber containing the kernel powder slowly fills with warm solvent. Some of the oil will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. The thimble ensures that the rapid motion of the solvent does not transport any kernel powder to the still pot. This cycle may be allowed to repeat many times, over hours or days.

During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled the advantage and disadvantage of soxhlet extractor is sumeriazed in table 2.5 (Cheahli chin, 2009).

After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted oil

Table 2.6 Advantage and disadvantage of soxhlet extractor

Advantage	Disadvantage
1. Long experience of use	1. Long extraction time (hours)
2. A displacement of transfer equilibrium occurs as the solid is continuously exposed to fresh solvent	2. Considerable solvent consumption
3. High extraction temperature enables exhaustive recovery of interest	3. Non selective extraction
4. Simple to operate	4. Risk of thermal decompositions as the extraction is conducted at the boiling point of the solvent
5. Economical	5. Only temperature and solvent type can be varied

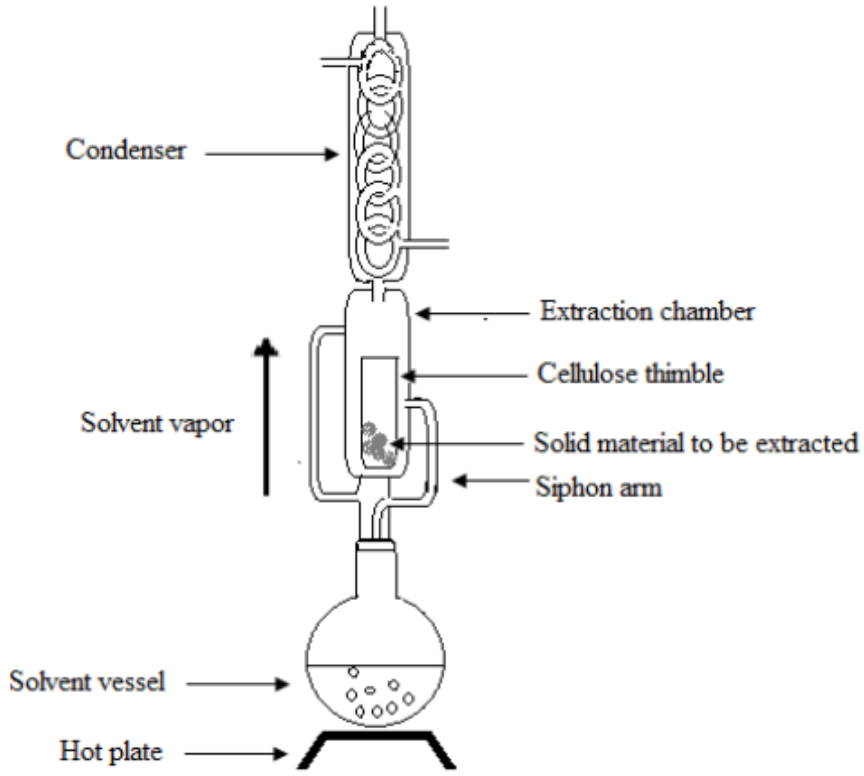

Figure 2.7 Soxhlet extractor

2.8.2.2. Factors affecting the rate of solvent extraction

The efficiency of solvent extraction of oil from oil seeds (mango seed kernel) can be influced by different factors such as particle size, solvent type used, temperature, extraction time, geographical variation that the seed is originated (Genetic variation), moisture content of the seed, solid to solvent ratio.

a) Particle size

Particle size influences the extraction rate in a number of ways. The smaller the size, the greater is the interfacial area between the solid and liquid, and therefore the higher is the rate of transfer of material and the smaller is the distance the solute must diffuse within the solid as already indicated. On the other hand, the surface may not be so effectively used with a very fine material

if circulation of the liquid is impeded, and separation of the particles from the liquid and drainage of the solid residue are made more difficult (Richardson et al., 2002).

Therefore, the use of a compromise particle size is almost invariably desirable. To obtain adequate oil release, particle diameters or thicknesses in the 0.2 - 5 mm range usually represent a good choice for industrial scale extractions (Henry, 1983)

b) Temperature

Temperature generally affects both the equilibrium and mass transfer rate of the extraction process. In the former, a higher temperature results in greater solubility of compounds in the solvent, resulting in a larger *K* value (equilibrium constant). In the latter, the higher the temperature, the higher will be the *D (*diffusion coefficient*),* hence increasing rate of extraction. In conventional solid-liquid (solvent) extraction processes, temperature is limited by the boiling point of the solvent. It is also important to bear in mind that increasing the extraction temperature may also potentially degrade thermolabile bioactive compounds. Thus, an optimized balance has to be determined when selecting the extraction temperature.(Cheahli- Chin, 2009)

The temperature of the extraction should be chosen for the best balance of solubility, solvent-vapor pressure, solute diffusivity, solvent selectivity, and sensitivity of product (Perry and Green, 1999)

c) Solvent Type

The liquid chosen should be a good selective solvent and its viscosity should be sufficiently low for it to circulate freely. Generally, a relatively pure solvent will be used initially, although as the extraction proceeds the concentration of solute will increase and the rate of extraction will progressively decrease, first because the concentration gradient will be reduced, and secondly because the solution will generally become more viscous (Richardson et al., 2002).

Most methods that use solvent extraction have used a trial and error approach. The Delaney amendment to the Food and Drug Act prohibits the use in food production of materials that exhibit any evidence of carcinogenicity. Therefore, even though great efforts are made to reduce solvent residues in products to extremely low levels, for example, parts per billion, many common solvents, such as benzene, and most chlorinated solvents, with the notable exception of methylene chloride, cannot be used in food processing aswell as Pharmaceuticals and cosmotics .

The most commonly used solvents for food processing are water, aqueous solutions of acids and nontoxic salts, commercial hexane, and in some cases other alkanes, ethanol and to a lesser extent the other lower alcohols, methylene chloride, methyl ethyl ketone, and acetone.

The use of alcohols and alcohol-water mixtures for extracting vegetable oil has attracted attention recently. These solvents can provide greater selectivity than hexane, which is currently used for most vegetable oil extractions. Alcohols and alcohol-water mixtures can also be separated from extracted oil more readily and with less expenditure of energy (Henry, 1983)

As is usually the case, it is desirable for solvents to be cheap, noncorrosive, nonflammable, nonexplosive,nontoxic, easily removable, and easily recoverable. It obviously may be impossible to meet all these objectives. The characteristics of the matrix to be extracted, mass transfer mechanisms also have to be considered in developing an optimal extraction system (Waldeback, 2005).

Since oil extracted from mango seed kernel has an application on cosmetics due to good source phenolic compound, and this phenolic compounds are highly dependent on solvent type used for the extraction. `

d) Time of extraction

In general, a prolonged extraction time results in an increased yield of the oil until equilibrium is reached. Thereafter, the concentration of compound will not increase further but there will have greater liability for degradation. Prolonged extraction time is also not desirable from an economic standpoint of labor and energy requirements. Therefore it is essential to find an optimum extraction time.

2.8.3 Supercritical fluid extraction

Supercritical fluid extraction (SFE) is the process of separating one component (the extract) from another (the matrix) using supercritical fluids as the extracting solvent. Extraction is usually from a solid matrix, but it can also be from liquids (Sapkale et al., 2010). A supercritical fluid is an element or compound above its critical pressure and temperature as shown in Fig 2.3. In this state, it is compressible and possesses both the properties of a gas and a liquid, providing the supercritical fluid with improved solvating power and has an edge over conventional liquids. The main attraction of SFE is the use of carbon dioxide (CO_2) as the solvent (extractant). Unlike most

organic solvents, CO_2 is not as harmful environmentally and has been described as a "green solvent". It is an inexpensive, relatively inert and non-inflammable gas of low toxicity, with easily achievable supercritical conditions (Tc =31.3°C, Pc = 73.8 bar). Supercritical CO_2 (SC-CO_2) has virtually no surface tension, thereby allowing better penetration into compared to liquid solvents. An added advantage is the possibility of conducting solventless extraction with SFE, as CO_2 would simply depressurize, subsequently depositing the extract into the collection vessel. However, CO_2 is a nonpolar fluid and has no permanent dipole moment. Thus, there is limited ability to dissolve polar or high molecular weight compounds (Wright et al., 1987)

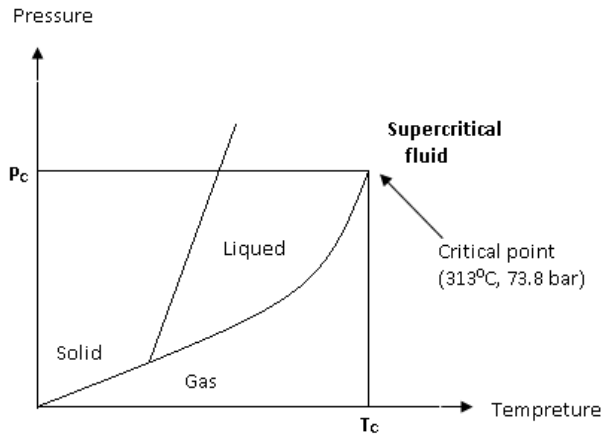

Figure 2.8 Phase diagram of carbon dioxide

Earlier applications of SFE processes include environmental and soil analyses and applications in the food industry. Most applications in the latter category include gravimetric analysis of fat from meat, and essential oils from spices. (Doane-Weideman et al., 2004). And oil and triglycerides from seeds (Boutin and Badens, 2009).

In the recent two decades, the range of applications has expanded to include extraction of high-value-added phytochemicals and nutraceuticals from natural products and medicinal plants.

Supercritical CO_2 extraction has been widely applied to the extraction of flavors, essential oils, antioxidants and bioactive principles from a variety of botanical matrices such as whole plants, seeds, roots, stems, leaves, flowers, fruits and peels. This technology has also attracted strong interest in the extraction and isolation of phenolics and antioxidants (Yesil-Celiktas et al., 2009)

Table 2.6 Advantage and disadvantage of SFE

Advantage	Disadvantage
1. Enhanced extraction efficiency	1. High capital investment
2. Tunability of the solvent strength	2. Large number of variables to optimize
3. Low organic solvent consumption	3. Strong dependence on matrix analyte interactions
4. Preservation of bioactive properties and organoleptic properties of the extracts	4. Difficulties in scale up and technology transfer
5. In- line interaction with sample preparation and detection methods	5. Difficulty in implementing continous extraction processes
	6. Difficulty of extracting more polar compounds

Chapter 3

Materials and Methods

The experimental work has been done in laboratory of Addis Ababa institute of technology school of chemical and bio Engineering and Science faculty Center of food science and nutrition of Addis Ababa University, Addis Ababa Ethiopia.

3.1 Materials and equipment

Materials used during the experiment were mango seed kernel, hexane (99.9%), petroleum ether (99%), ethanol (99.9%), sodium hydroxide (99%), potassium hydroxide (85%), hydrochloric acid, folin ciocalteu reagent, Gallic acid, saturated sodium carbonate, acetone, phenolphthalein, filter paper, distilled water. All the chemicals and reagents were purchased and obtain from Wise team PLC, school of chemical and bioengineering of Addis Ababa institute of technology, center of food science and nutrition of science faculty of Addis Ababa University.

The equipments used were soxhlet extractor, vacuum pump, chiller, water bath, centrifuge, condenser, oven, vibro viscometer, flask, beaker, balance, dissector, test tubes, UV- Visible spectrophotometer, sieve, density bottle, centrifugal miller.

3.2 Experimental Methods

3.2.1 Raw material preparation

Assosa mango fruit (local variety) was purchased from Ethiopian Etfruit company, kenuma and Akea small scale juice processing house. The peel and the flash part were removed by hand in order to obtain the seed. The seed were dried by sun for five days. The hard cover of the seed was decorticated manually to obtain the kernel. The kernel was then dried in the oven at 50^0C for 18 hour.

3.2.2 Determination of moisture content of the kernel

Seven sample of the kernel randomly were weighed and dried in an oven at 105^0C and the weight was measured every two hours. The procedure was repeated until a constant weight was obtained and the percentage moisture content of the kernel was determined.

3.2.3 Size reduction and sieve analysis

After the moisture was removed by placing in an oven at 50^0C for 18 hours the dried mango seed kernel was milled in centrifugal miller with a sieve size of 4mm.Then the sample was sieved using vibrating shaker for 15 minutes with amplitude of 5mm. The sieve size was arranged in descending order of mesh size 4mm, 3mm, 1.5mm, 1mm, 0.75mm, 0.5mm and 0.25mm to obtain a particular size of 3 – 1.5mm, 1.5 – 0.5mm and 0.5 – 0.25mm. This particular size range was selected because literature reveled that to have a higher yield of oil the particle size should be less than 5mm and higher than 0.2mm (Henry, 1983).

3.3 Oil Extraction

The oil extraction was conducted using soxhlet extractor in triplicate with three different solvents: hexane, petroleum ether and ethanol. The solvents chosen for this study are normally used to extract oil from plant kernel and also it is reported that hexane and petroleum ether were petroleum based solvents and highly toxic and carcinogenic where as ethanol is non toxic and can be made from plant part, it is called "green solvent" so to compare their efficiency both on oil yield and phenolic compound extraction and also to see the opportunity of ethanol to be used as a solvent, these solvents were selected. Seventy five gram of mango seed kernel with three different particle size: 3 – 1.5mm, 1.5 -0.5mm and 0.5 – 0.25mm were fed to a soxhlet extracter with 300ml solvents for three different time: 2 hour, 4 hour and 6 hour, these values were selected based on previous results for similar seed (Nwabanne, 2012; Lawson et al., 2010; Tunmis et al., 2012). Extraction temperature of 50 ^{0}C was chosen to avoid thermal degradation of bioactive compounds like phenolic compounds in the extract and also the temperature is in the range of boiling point of the solvents (Kittiphoom and Sutasinee, 2013). The resulting extracts, obtained under different operating conditions were separated by evaporating the solvents using simple distillation in which the setup was established in the laboratory under reduced pressure and temperature of 50^0C. The products were weighted and the oil physicochemical properties were determined.

3.3.1 Determination of percentage of oil extracted

The percentage yield was calculated in two forms i.e oil yield and extraction yield using the formula below.

$$\text{pecentage oil yield} = \frac{\text{mass of oil}}{\text{mass of oil precent in the seed}} * 100\%$$

Since mango seed kernel have an oil content of 8-16%, an average 12% oil content was taken for calculating the yield therefore:

$$\text{percentage oil yield} = \frac{\text{mass of oil}}{0.12 * \text{mass of the sample}} * 100\% \qquad (3.1)$$

The second form that the yield calculated was

$$\text{percentage extraction yield} = \frac{\text{mass of oil}}{\text{mass of the sample}} * 100\% \qquad (3.3)$$

3.4 Characterization of the Extracted Oil

The acidity, iodine, peroxide and saponification values are the major characterization parameters for oil quality. Physical properties like moisture content (volatile matter), specific gravity, viscosity, PH value and refractive index and chemical properties like saponification value, unsaponifiable matter, iodine value, acid value, peroxide value and total phenolic content were determined for the oil which was extracted using optimum operating parameters.

3.4.1 Characterization of the Physical Properties of Oil

3.4.1.1 Determination of Moisture and volatile matter of oil

5 gm of oil was weighted and putted in a dish and then dried in an oven at 105 ^{0}C for 1 hour. The dish was removed from the oven and cooled in a dissector and weighed. The process was repeated until a constant weight was observed and the moisture and volatile matter of the oil was determined (Hand book of food analysis, 1984).

3.4.1.2 Determination of the specific gravity

The density of oil was determined using density bottle method. A clean and dry density bottle of 25ml capacity at 30^{0}C was weighed in gm. Then the bottle was filled with water and reweighed at 30^{0}C. Melted oil was brought to 30^{0}C and the water was substituted with this oil after drying the density bottle and weighed again and the specific gravity was determined (A.O.A.C official method 920.212, 2000).

3.4.1.3 Determination of kinematic viscosity of oil

A kinematic viscosity of the oil was measured indirectly using vibro viscometer which is available in laboratory of school of chemical and bio engineering. Initially, a sample was heated at a temperature of 30^{0}C. A sample of 35 ml oil was measured and fed to a sample holder of the vibro viscometer. A sensor of the viscometer was immersed to the oil and then a dynamic viscosity of oil was displayed on the vibro viscometer screen at a temperature of 30^{0}C. And then the kinematic viscosity was calculated.

3.4.1.4 Determination of PH

2 gm of the sample was taken and putted in to a clean dry 25 ml beaker and 13 ml of hot distilled water was added to the sample in the beaker and stirred slowly. And then it was cooled in a cold

water bath to 25^0C. The PH electrode was standardized with a buffer solution first and then immersed in to the sample and the PH was read (A.O.A.C, Official method of analysis 960.19, 2000).

3.4.1.5 Determination of refractive index

The refractive index of the sample was determined using refractometer at JIJE analytical testing service laboratory plc using A.O.A.C official method 921.08

3.4.2 Characterization of the Chemical Property of Oil

3.4.2.1 Determination of Saponification value

2 gm of the sample was taken and added in to 250 ml flask. 25 ml of alcoholic potassium hydroxide solution was added in to the flask. The flask was connected to reflux condenser and kept on the water bath and boiled gently for 1 hour. After the flask and the condenser were cooled, the inside of the condenser was washed with 10 ml of hot ethyl alcohol. Then few drops of phenolphthalein indictor were added and the excess potassium hydroxide was titrated with 0.5 N hydrochloric acid to the end point, until the pink color of the indictor just disappears. The same procedure was conducted for the blank and the saponification value (SV) experesed as the number of milligrams of KOH required to saponify 1 gm of fat was calculated (A.O.A.C official method 920.160, 2000).

3.4.2.2 Determination of unsaponifiable matter

5 gm of the sample was taken and added in to 250 ml conical flask. 50 ml of alcoholic potassium hydroxide solution was added and the content was boiled under a reflux condenser for one hour. The condenser was washed with 10 ml of ethyl alcohol. The saponified mixture was transferred to separating funnel and it was allowed to cool to 25^0C. After 50 ml of petroleum ether was added in to the separating funnel and shook vigorously, it was allowed the layers to separate. The lower soap layer was transferred in to another separating funnel and the ether extraction was repeated three times using 50 ml portion of petroleum ether. To insure the ether extract was free of alkali, the combined ether extract was washed three times with 50 ml aqueous alcohol followed by 25 ml distilled water. The ether solution was transferred to 250 ml beaker and the ether was evaporated. When all the ether had been evaporated 3 ml of acetone was added while on the water bath to remove the solvent completely under gentle air. To remove the last traces of

the ether it was dried in un oven at 100^0C for 30 minutes. Then the residue was dissolved in 50 ml of warm ethanol and titrated with 0.02N sodium hydroxide. And then the unsaponifiable matter was determined (A.O.A.C official method 933.08, 2000).

3.4.2.3 Determination of iodine value

The iodine value of the sample was determined by using A.O.A.C official method 993.20 iodine value of oil and fat at JIJE Analytical testing service laboratory plc.

3.4.2.4 Determination of acid value

2.5104 gm of the sample was weighed and putted in to 250 ml conical flask and 50 ml of hot ethyl alcohol was added to the flask. After few drops of phenolphthalein was added, the mixture was boiled for about five minutes and while it was hot titrated with 0.5N sodium hydroxide solution and then the acid value was determined. Since the acidity is frequently expressed as free fatty acid from acid value free fatty acid was calculated.

3.4.2.5 Determination of peroxide value

The peroxide value of the sample was determined at JIJE Analytical testing service laboratory plc using A.O.A.C official method 965.33 peroxide value of oil and fat.

3.4.2.6 Determination of total phenolic content

The phenolic compounds concentration in mango seed kernel oil ethanoic, hexanoic, and petroleum ether extracts was determined using folin – ciocaletea method based on the procedure described by Brand, Cuveliner, and Brest. 1995, 0.4 ml of mango kernel oil were mixed with 2 ml of 10% Folin ciocaltea reagent and 1.6 ml of 7.5% Na_2Co_3 and left at room temperature in a dark place for 30 minutes. The mixing solution was measured an absorbance at 965nm by ultraviolet visible spectrophotometer. A blank sample consisting of water and reagent was used as a reference. The results were expressed as milligrams of galic acid equivalents per gram of the sample (mg GAE/g sample) by reference to galic acid calibration curve.

3.5 Design of the Experiment

3.5.1 Full Factorial design

Factorial design is used to investigate the effect of each factor. In a factorial experiment all the possible combinations factor levels would be tested and it would be possible to determine the effect of individual factors and to assess the effect of change of two or more variable at a time (Zivorad, 2004).

The analysis was performed by utilizing Design Expert soft ware using general factorial design method. This method of experiment design helps to differentiate the significance of the main and the interaction factors. The soft-ware also used to develop the mathematical model that will describe the effect of main and interaction factors on the Response.

Factors: 3 factors were investigated as mentioned earlier; these were: particle size, time of extraction and solvent type.

Factors Levels: For each factors, three levels were considered.

Replicates: Each independent experiment was repeated three times.

Number of runs: For "m" levels, "n" factors and "k" replicate, the number of experimental runs that need to be performed is equal to $\mathbf{k*m^n}$ (Zivorad, 2004). In this research m=3, n=3 and k=3 thus, $\mathbf{3*3^3 = 81}$ experimental runs were performed.

The factors and their levels

The levels that were selected for each factors are:

1. Particle size (mm): 0.25-0.5, 0.5-1.5 and 1.5-3.0

2. Extraction time (hr): 2, 4 and 6

3. Solvent type: hexane, ethanol and petroleum ether

Model equation

Finally, Regression models were established for the dependent variables to fit the experimental data for the response using Design expert 7.0.0 soft ware.

Chapter Four

Results and Discussion

4.1 Moisture content of the seed kernel

Fresh mango fruit was collected on February 2014, after the flesh part was removed and the seeds were dried by the sun for 3 days it was decorticated and by taking seven samples (11.6, 16.2, 10.4, 10.5, 9.5, 4.9, 3.5 gram) of the mango seed kernel, the moisture content of the samples were determined using equation (AG.1) and summarized in table 4.1.

Table 4.1 Moisture content of Mango seed kernel

	Sample weight in gram						
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Initial mass	11.6	16.2	10.4	10.5	9.5	4.9	3.5
Mass after 2 hrs	8.6	12.7	7.4	8.1	6.5	3.4	2.1
Mass after 4 hrs	6.7	9.8	5.6	6.3	4.9	2.9	1.9
Mass after 6 hrs	6.3	9.0	5.4	5.8	4.6	2.6	1.8
Mass after 8 hrs	6.2	8.6	5.3	5.5	4.5	2.4	1.7
Mass after 10 hrs	6.2	8.6	5.3	5.5	4.5	2.4	1.7
Final moisture content	46.6%	46.9%	49.0%	47.6%	52.6%	51.0%	51.4%

The moisture content of the seven seed kernel samples having mass of 11.6, 16.2, 10.5, 10.4, 9.5, 4.9, and 3.5 grams were 46.6, 46.9, 49.0, 47.6, 52.6, 51.0 and 51.4 percent respectively. The mean plus the standard deviation of the seven samples gives 49.3% ± 2.47. The result obtained is

in agreement to those of the literature since (Dhingra and Kapoor 1985) and (Nzikoun et. al 2010) who reported a moisture content of 50.94% and 45.2% respectively.

4.2 Oil Extraction

4.2.1 Percentage Yield of Extraction

The percentage oil yield and percentage extraction yield was calculated by using equation 3.1 and 3.2 respectively and the result is summarized in the table 4.2 below.

Table 4.2 Percentage oil yield of Mango seed kernel oil for three factors, three levels and three replicas full factorial design

No of runs	Factors			Oil yield (%)				Extraction yield (%) Mass of oil extracted/ mass of seed kernel
	Particle size	Time	Solvent type	Replicate 1	Replicate 2	Replicate 3	Mean±SD	
1	0.25-0.5	2	Hexane	57.78	60.0	54.44	57.41±2.80	6.89±0.34
2	0.5-1.5	2	Hexane	43.33	45.56	44.44	44.44±1.12	5.33± 0.13
3	1.5-3	2	Hexane	42.22	40.0	44.44	42.22±2.22	5.07±0.27
4	0.25-0.5	4	Hexane	82.22	83.33	81.11	82.22±1.11	9.87±0.13
5	0.5-1.5	4	Hexane	58.89	57.78	58.89	58.52±0.64	7.02±0.08
6	1.5-3	4	Hexane	45.56	43.33	5.73	45.57±2.23	5.47±0.27
7	0.25-0.5	6	Hexane	84.44	86.67	83.33	84.81±1.70	10.18±0.2
8	0.5-1.5	6	Hexane	68.89	70.0	71.11	70.0±1.11	8.4±0.13
9	1.5-3	6	Hexane	64.44	64.44	63.33	64.07±0.64	7.69±0.08
10	0.25-0.5	2	Ethanol	23.33	22.22	25.56	23.70±1.70	2.84±0.20
11	0.5-1.5	2	Ethanol	21.11	20.0	20.0	20.37±0.64	2.44±0.08
12	1.5-3	2	Ethanol	17.78	20.0	18.89	18.89±1.11	2.27±0.13
13	0.25-0.5	4	Ethanol	48.89	47.78	50.0	48.89±1.11	5.87±0.13
14	0.5-1.5	4	Ethanol	40.0	42.22	41.11	41.11±1.11	4.93±0.13
15	1.5-3	4	Ethanol	30.0	30.0	31.11	30.37±0.64	3.64±0.08
16	0.25-0.5	6	Ethanol	56.67	55.57	58.89	57.04±1.69	6.84±0.20

17	0.5-1.5	6	Ethanol	46.67	46.67	47.78	47.04±0.64	5.64±0.08
18	1.5-3	6	Ethanol	43.33	44.44	47.78	45.18±2.32	5.42±0.28
19	0.25-0.5	2	Petroleum ether	62.22	61.11	61.11	61.48±0.64	7.38±0.03
20	0.5-1.5	2	Petroleum ether	38.89	40.0	36.67	38.52±1.70	4.62±0.20
21	1.5-3	2	Petroleum ether	40.0	40.0	40.0	40.0±0	4.8±0
22	0.25-0.5	4	Petroleum ether	73.33	75.56	73.33	74.07±1.29	8.89±0.15
23	0.5-1.5	4	Petroleum ether	56.61	56.67	57.78	57.02±0.66	6.84±0.08
24	1.5-3	4	Petroleum ether	44.44	42.22	45.57	44.08±1.70	5.29±0.20
25	0.25-0.5	6	Petroleum ether	83.33	82.22	84.44	83.33±1.11	10.0±0.13
26	0.5-1.5	6	Petroleum ether	68.89	71.11	67.78	69.26±1.70	8.31±0.20
27	1.5-3	6	Petroleum ether	61.11	60.0	63.33	61.48±1.70	7.38±0.20

From table 4.2 the maximum percentage oil yield obtained was 84.81±1.70 (which is equivalent to 10.18±0.2 percentage extraction yield) at particle size range of 0.25-0.5mm, for the extraction time of 6 hour and using hexane, where as the minimum percentage oil yield was 18.89±1.11 (which is equivalent to 2.27±0.13 percentage extraction yield) obtained at particle size range of 1.5-3mm, for the extraction time 2 hour and using ethanol as solvent.

A percentage extraction yield of 8.46±0.1 (which is equivalent to 70.5±0.83 percentage oil yield[1]) was reported by Kittiphoom and Sutasinee (2013) using hexane as a solvent with extraction time of 6 hour. Saipraha et al (2011) reported a yield of 10.2% (85% oil yield[1]) using hexane as a solvent and with extraction time of 5 hour. Additionally Nzikou et al (2010) reported a percentage extraction yield of 13.0 with hexane as a solvent and extraction time of 8 hour and a percentage extraction yield of 12.5±0.2 was reported by Maryam Fahimdanesh et al (2013).

[1] It is converted by combining equation (3.2) and (3.3)

The result obtained from this study was almost similar to the reported values and the smaller deviations may come from, variety difference of the mango seeds taken for the study and solid to solvent ratio taken for the extraction, extraction temperature and other may be the cases.

Optimization

Using optimization function in design expert soft ware 7.0.0, it was predicated that at the following operating condition; 0.39mm particle size, 5.67 hour extraction time and hexane as a solvent, a maximum oil yield of 85.01% was obtained which is in agreement with the experimental value 84.81%. A minimum yield of 16.987% was predicated at particle size 1.40mm, 2.22 hour extraction time and solvent type ethanol, which was also in agreement with the experimental value.

From design expert soft-ware of numerical optimization tool, the optimized solutions for maximum and minimum yield is shown in table 4.3 and 4.4 below.

Table 4.3 Solution output from numerical optimization for maximum oil yield

Number	Particle size	Extraction time	Solvent type	Yield	Desirability	Remark
1	0.4	5.76	hexane	85.1281	1	**Selected**
2	0.41	5.85	hexane	85.2479	1	
3	0.42	5.97	hexane	85.5787	1	
4	0.41	5.83	hexane	85.0278	1	
5	0.39	5.97	hexane	86.4244	1	
6	0.38	5.78	hexane	85.725	1	
7	0.39	5.92	hexane	86.3728	1	
8	0.41	5.8	hexane	84.9202	1	
9	0.41	5.77	hexane	85.0093	1	
10	0.4	5.93	hexane	86.0458	1	
11	0.39	5.83	hexane	85.8744	1	
12	0.4	5.88	hexane	85.6977	1	
13	0.4	5.83	hexane	85.471	1	
14	0.43	5.97	hexane	85.2938	1	
15	0.39	5.69	hexane	85.1206	1	
16	0.42	5.86	hexane	84.9615	1	
17	0.39	5.81	hexane	85.628	1	
18	0.4	5.91	hexane	85.7305	1	
19	0.39	5.79	hexane	85.559	1	

20	0.4	5.95	hexane	86.0811	1	
21	0.43	5.93	hexane	85.1359	1	
22	0.38	6	petroleum ether	84.6208	0.997	
23	0.38	5.95	petroleum ether	84.3725	0.993	
24	0.46	6	hexane	84.2998	0.992	
25	0.38	5.55	petroleum ether	82.3699	0.963	
26	2.25	6	hexane	60.7815	0.635	
27	0.38	6	ethanol	59.8197	0.621	
28	2.25	6	petroleum ether	58.3925	0.599	
29	2.25	6	ethanol	44.8373	0.394	

Table 4.4 Solution output from numerical optimization of design expert soft ware for minimum oil yield

Number	Particle size	Extraction time	Solvent type	Yield	Desirebility	Remark
1	1.83	2.12	ethanol	16.1834481	1	**Selected**
2	2.1	2.21	ethanol	18.82158	1	
3	1.67	2.06	ethanol	15.2771702	1	
4	1.7	2.03	ethanol	15.0139423	1	
5	1.78	2.12	ethanol	16.0305557	1	
6	1.85	2	ethanol	15.2880407	1	
7	1.87	2.1	ethanol	16.237851	1	
8	1.82	2.16	ethanol	16.5260289	1	
9	1.37	2.1	ethanol	16.0630302	1	
10	1.2	2.11	ethanol	17.2221131	1	
11	1.96	2.03	ethanol	16.1219296	1	
12	1.55	2.35	ethanol	17.7787049	1	
13	1.45	2.28	ethanol	17.3372928	1	
14	1.83	2.05	ethanol	15.5937607	1	
15	1.34	2.28	ethanol	17.8218228	1	
16	1.73	2.34	ethanol	17.7448396	1	
17	1.75	2.33	ethanol	17.7380073	1	
18	1.71	2.3	ethanol	17.3550389	1	
19	1.59	2.4	ethanol	18.1649782	1	
20	1.77	2.19	ethanol	16.6222466	1	
21	1.93	2.16	ethanol	17.0946698	1	
22	1.85	2.06	ethanol	15.7597594	1	
23	1.44	2.24	ethanol	17.04251	1	
24	1.56	2.09	ethanol	15.4799508	1	
25	1.58	2.36	ethanol	17.8416724	1	

4.3 Effect of process parameters in percentage oil yield

4.3.1 Effect of particle size on percentage oil yield

The effect of particle size for a particle size range of 0.25-0.5mm, 0.5-1.5mm and 1.5-3mm on oil yield for hexane as a solvent, 2 hour, 4 hour and 6 hour extraction time is shown figure 4.1.

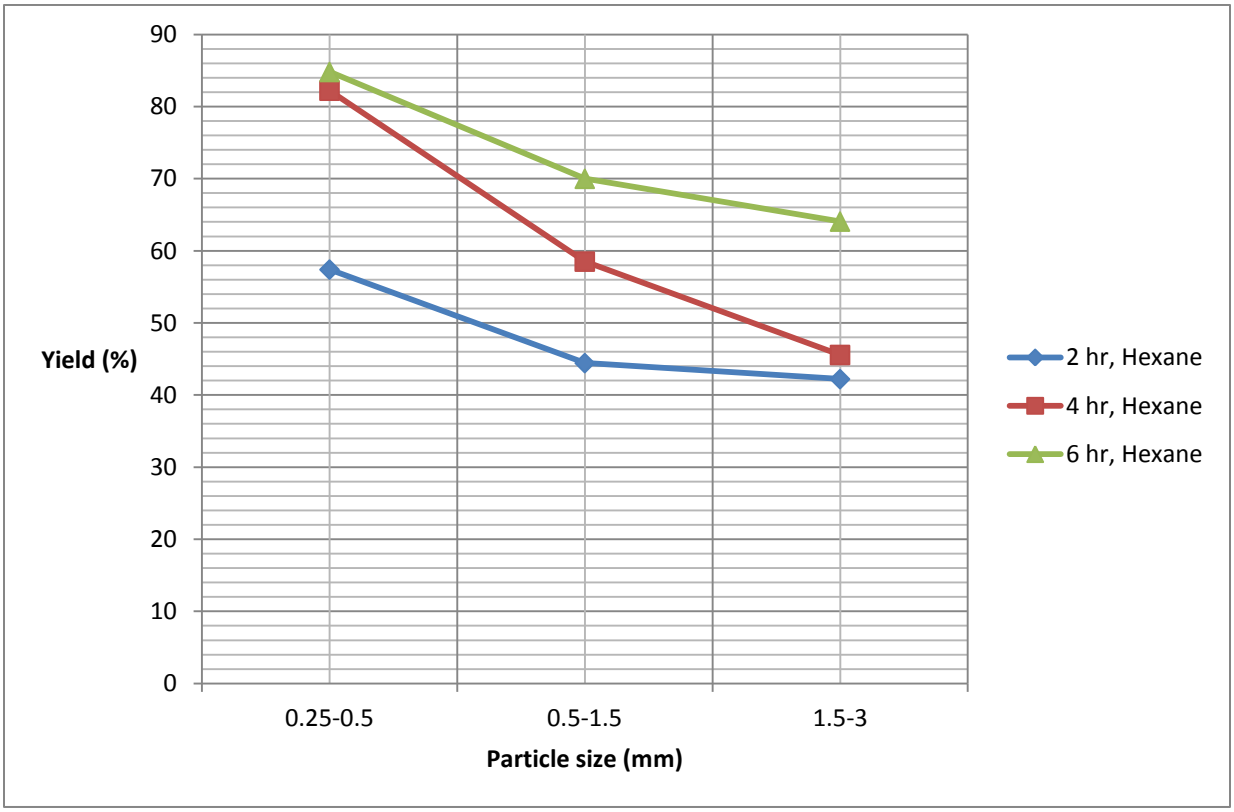

Figure 4.1 Effect of particle size on percentage oil yield for three different extraction time and hexane as a solvent

The particle size plays a great role on yield of mango seed oil (figure 4.1). The percentage oil yield was inversely related to the particle size i.e smaller particle size gives high yield while larger particle size results a lower yield. For 2 hr, 4 hr and 6 hr extraction time as a particle size decreases from particle size range of 1.5-3mm to 0.25-0.5mm the oil yield increases from 42.22% to 57.41%, 45.57% to 82.22% and 64.07% to 84.81% respectively i.e the yield of smaller particle size is 15.2% for 2 hr extraction, 36.7% for 4 hr extraction and 20.7% for 6 hr extraction time higher than that of larger particle size. The reason is that larger particles have

smaller surface area of contact and a greater distance to solvent entrance and oil diffusion in comparison to smaller particles (Ebewele et al., 2010).

However, when the particle size is too small or very fine, the oil yield decreases the reason may be due to agglomeration of fine particle which reduces the contact surface area.

Spider Sayyar et al 2009 reported the result of extraction of oil from jatropha seed for three different particle size ranges < 0.5mm, 0.5-0.75mm and > 0.75mm. The highest percentage of oil yield was obtained with the intermediate particle size (0.5-0.75mm) which indicated that decreasing the particle size below a certain particle size doesn't increase the percentage of oil yield and may even decrease the yield. Thus, we can conclude that decreasing the particle size below 0.25mm may decrease the oil yield.

4.3.2 Effect of extraction time in percentage oil yield

The effect of extraction time on oil yield for solvent type hexane and particle size range of 0.25-0.5mm, 0.5-1.5mm and 1.5-3mm is shown in figure 4.2.

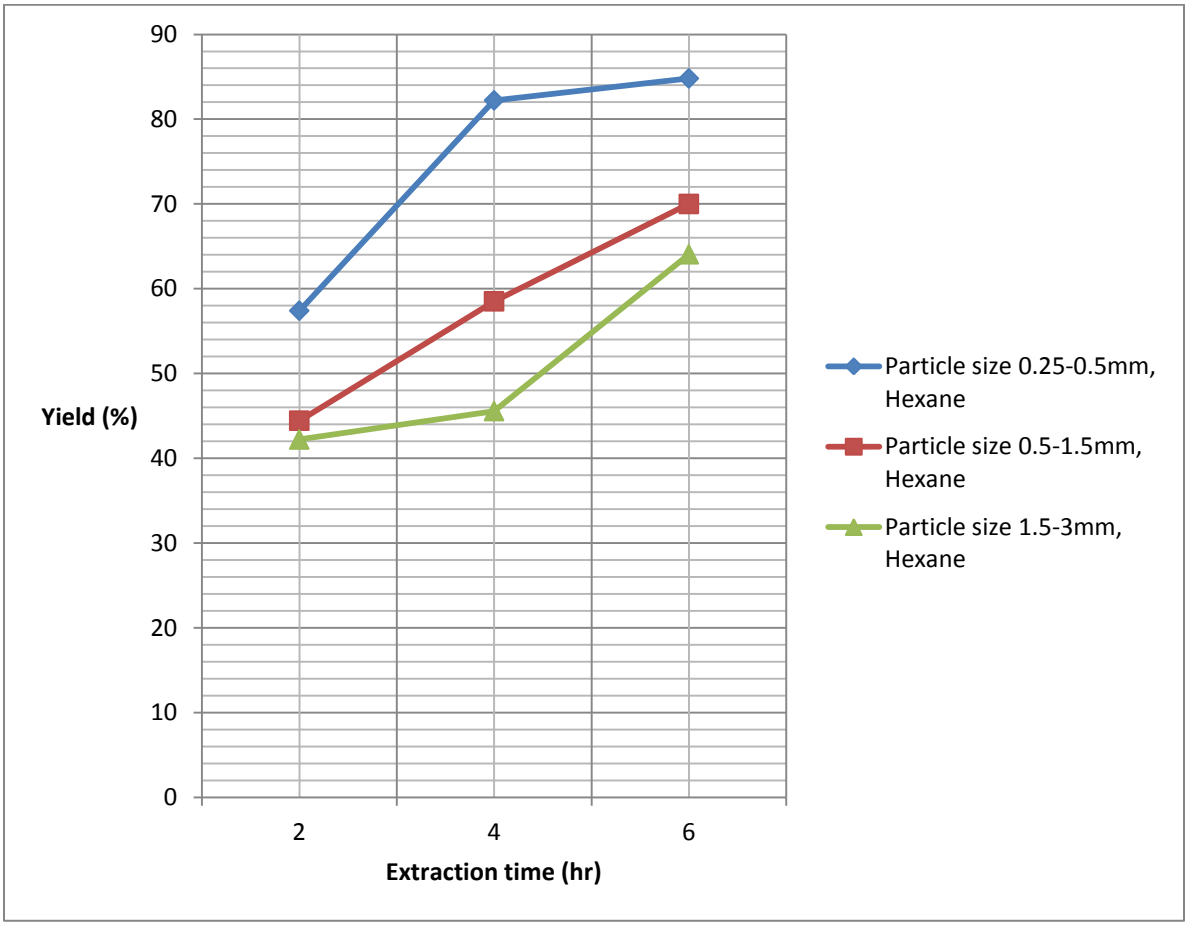

Figure 4.2 Effect of extraction time on oil yield

The percentage oil yield was directly related to extraction time i.e the yield increased as extraction time increased (figure 4.2). The same trend was reported by Meziane et al (2008) and Stanisarejevic et al (2007)

For smaller particle size range 0.25-0.5mm the yield rose rapidly with time up to 4 hour and thereafter the yield of oil was not varying (it was constant). At 4 hour the oil in the seed was almost exhausted hence negligible oil yields. The oil yield increased by 24.81% as the extraction time increased from 2 hour to 4 hour and it increased by only by 2.59% as the time increased from 4 hour to 6 hour. However for larger particle size i.e 1.5-3mm the yield was lower at the

beginning of the extraction and increased gradually as the extraction time increased. The yield increased by only 3.35% as the time increased from 2 hour to 4 hour while by 18.50% as the extraction time increased from 4 hour to 6 hour.

The result obtained in this research indicates that smaller particle size needs small extraction time to obtain maximum yield in comparison to large particle size. According to this study the maximum oil yield is obtained at 6 hrs extraction time and at lower particle size and since at 4 hours extraction time 97% of the maximum yield was obtained, so extraction time above 6 hrs is wastage of time and cost. Thus, the extraction should be stopped after 6 hrs of extraction.

4.3.3 Effect of solvent type on percentage oil yield

The effect of solvent type (hexane, ethanol and petroleum ether) for particle size range 0.25-0.5 and extraction time 2 hour, 4 hour and 6 hour on oil yield is shown in figure 4.3.

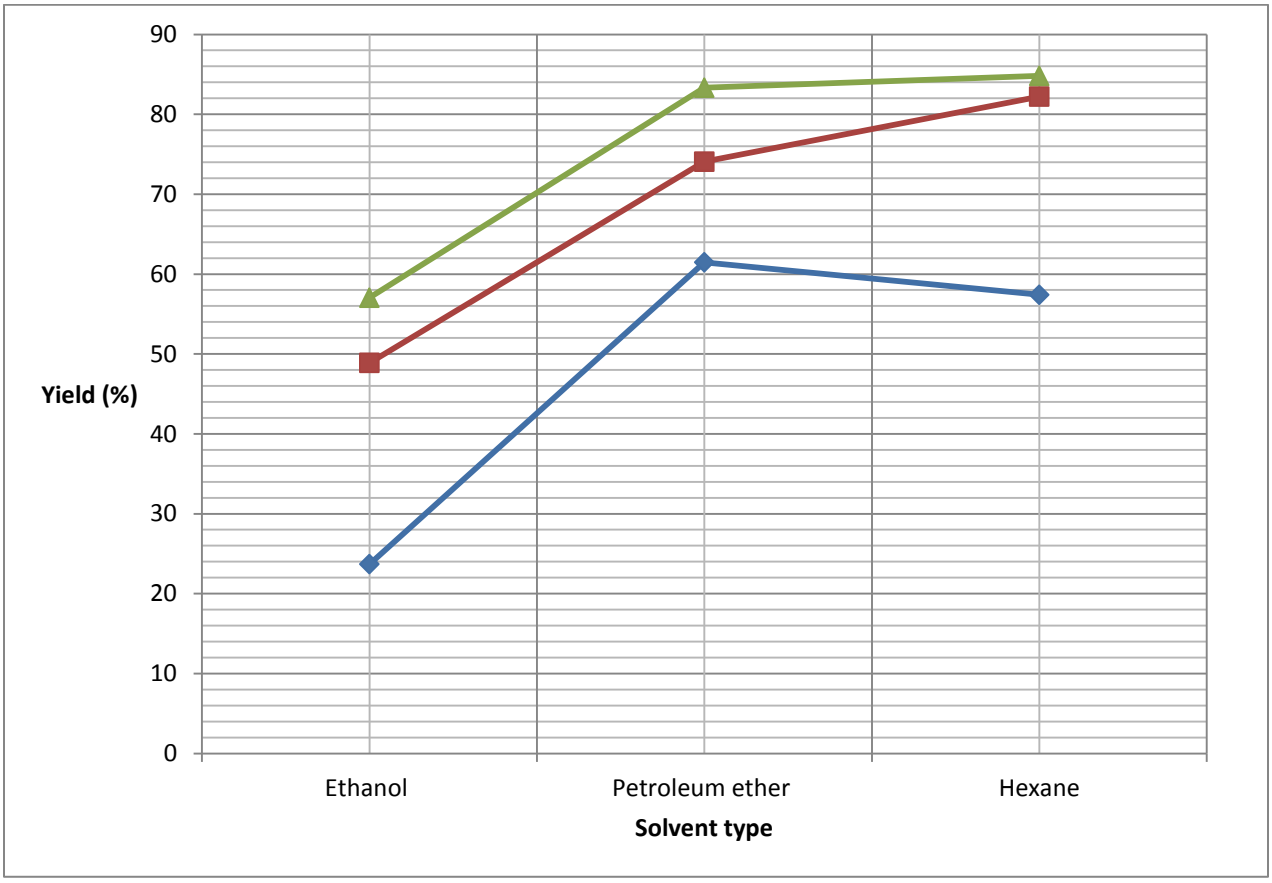

Figure 4.3 effect of solvent type on oil yield for particle size range of 0.25-0.5mm and for three different extraction times 2, 4 and 6 hr

Considering figure 4.3 for a particle size range of 0.25-0.5mm, the maximum oil yield using hexane as a solvent was found to be 84.81% at extraction time of 6 hour and the minimum yield was 57.41% at extraction time of 2 hour. The maximum and minimum yield for the same operating conditions but using petroleum ether as a solvent was found to be 83.33% and 61.48% respectively while ethanol solvent resulted a maximum yield of 57.04% and minimum yield of 23.70%. Kittiphom and Sutasinee (2013) reported 8.46, 8.04 and 6.96 percentage extraction yield

(which is equivalent to 70.5%, 67.0% and 58.0% extraction yield[2]) using hexane, petroleum ether and ethanol as a solvent respectively for 8 hour extraction time and unknown particle size.

According to the result obtained in this study, the maximum percentage oil yield of petroleum ether (83.33%) was almost equal to that of hexane (84.81%). It is known that the boiling point range of petroleum ether (40^0C – 60^0C) is lower than that of hexane (65^0C -69^0C) so to avoid thermal degradation of bioactive components it is preferable to use petroleum ether than hexane.

The yield obtained from ethanol was the lowest in comparison to hexane and petroleum ether. This is because since both hexane and petroleum ether are non polar organic solvents they have high capacity to dissolve non polar compounds while ethanol can extract non oil components due to the presence of OH bond (polar). However currently both hexane and petroleum ether are obtained from petrochemical sources these solvents can be emitted to the atmosphere during extraction and recovery and have been identified as an air pollutant since it can react with other pollutants to produce ozone and photochemical oxidants(Wan et al., 1995; Hamoungjai et al., 2000)

Safty, environmental and health concerns have increased the interest in alternative solvents to hexane to reduce the emission of volatile organic compound to atmosphere as well as potential trainces in oil after reefing.

Ethanol is a worthy candidate to investigate as an alternative solvent since its cost is low and it may be produced from a large variety of biological materials using simple technology. In addition, although flammable (flash point= 8.9^0C; ignition temperature= 425^0C), this alcohol is recognized as non-toxic and has less handling risks than hexane (flash point = -23^0C; ignition temperature= 225^0C) . It can also be obtained by fermentation and therefore labeled as ‘‘natural’’. The use of this alcohol as an extraction solvent also avoids eventual toxicity problems of meals for animal feedstuff (Suzana et al., 2003)

Therefore, even if the yield obtained from ethanol was the lowest, from safety, health and environmental point of view it is better to use ethanol as a solvent than hexane petroleum ether.

[2] It is converted by using equation (3.2) and (3.3)

4.3.4 Interaction effect among the three factors

From design expert soft ware 7.0.0 output, interaction effect between;

- Particle size and extraction time for solvent type hexane
- Solvent type and particle size for extraction time of 6 hours and
- Extraction time and solvent type for particle size range of 0.25-0.5mm

On percentage oil yield are shown in figure below.

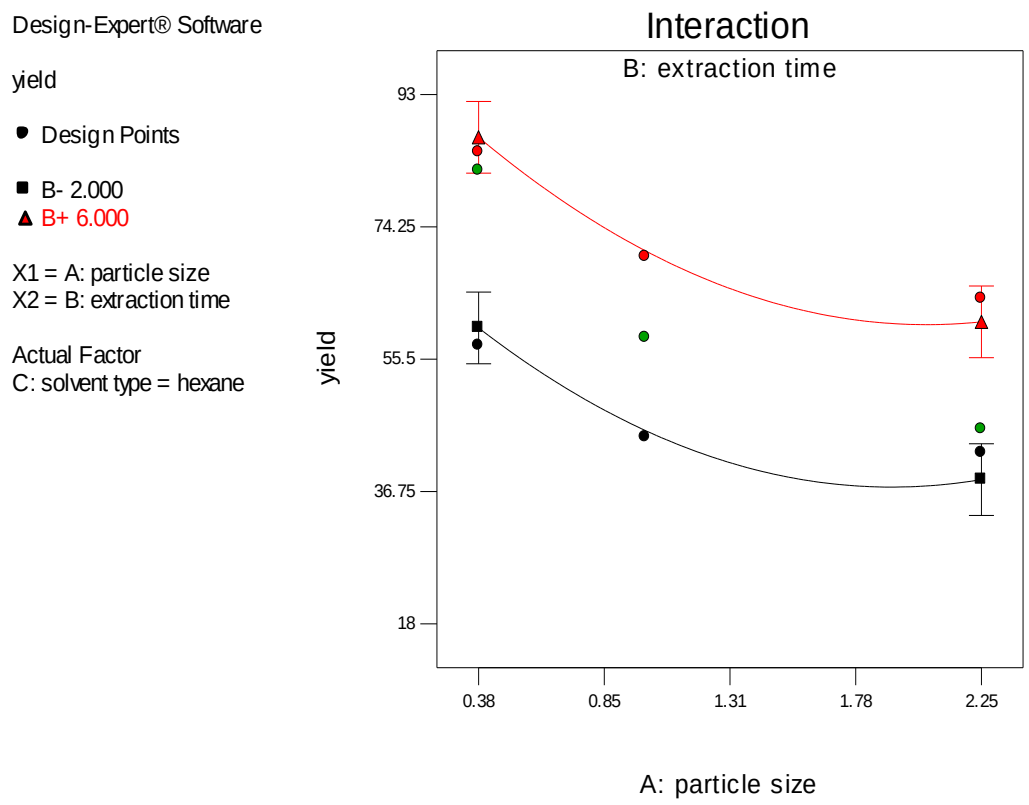

Figure 4.4 Interaction effect of particle size and extraction time on oil yield

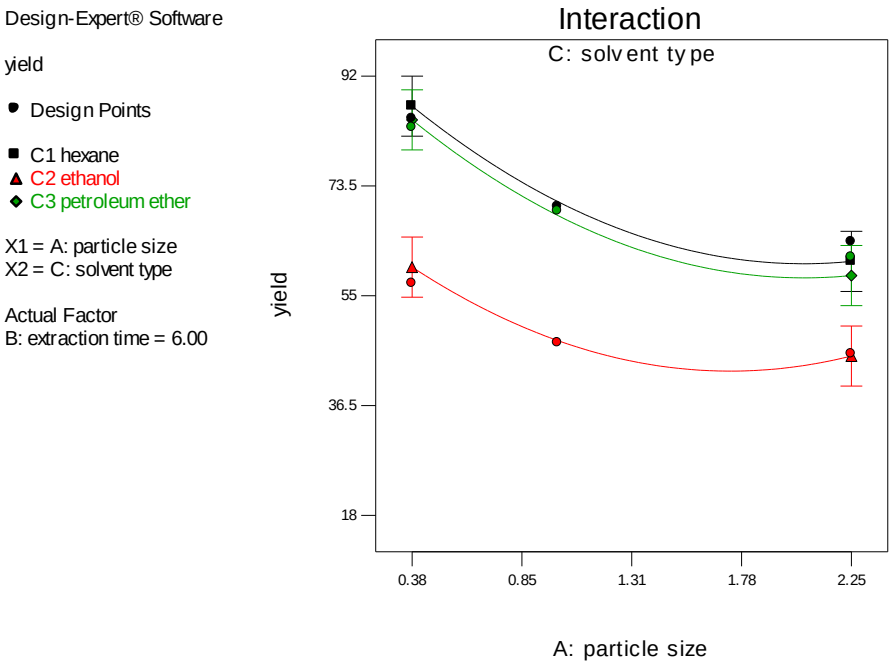

Figure 4.5 Interaction effects of particle size and solvent type on oil yield

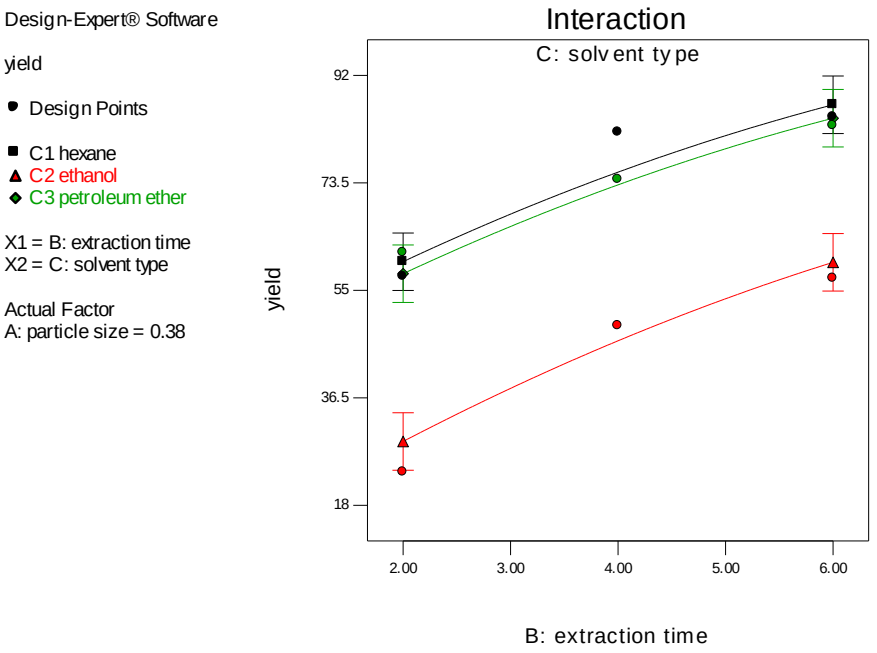

Figure 4.6 Interaction effects of extraction time and solvent type on oil yield

There was no interaction between particle size and time as depicted by similar shape of the two curves in the figure 4.4. This implies that irrespective of extraction time, lower particle size can give a higher yield and irrespective of particle size, higher extraction time can give higher yield. Similarly as can be noticed from figures 4.5 and 4.6 there was no interaction effect between solvent type and particle size and between extraction time and solvent type respectively.

Regressional model equation

The following table shows analysis of variance (ANOVA) obtained from Design expert soft ware, which tells as the significance of different factors.

Table 4.5 Analysis of variance (ANOVA) table for a response of percentage oil yield of mango seed kernel

Source	Sum of squares	Degree of freedom	Mean square	F value	P value Prob > F
Model	8362.18	11	760.20	35.57	< 0.0001
A-particle size	16022.12	1	1602.12	77.07	< 0.0001
B-Extraction time	3072.93	1	3072.93	147.83	< 0.0001
C-solvent type	3185.37	2	1592.68	76.62	< 0.0001
AB	16.91	1	16.91	0.81	0.38
AC	130.38	2	65.19	3.14	0.0728
BC	15.68	2	7.84	0.38	0.6921
A^2	316.85	1	316.85	15.24	0.0014
B^2	21.94	1	21.94	1.06	0.3205
Residual	311.81	15	20.94		
Cor Total	8673.99	26			

The model F- value of 35.57 implies the model is significant. Value of "prob>F" less than 0.05 indicates the terms are significant. In this case A-particle size, B-extraction time, C-solvent type and A^2-square of particle size are significant model terms. Values greater than 0.1000 indicates the model terms are not significant. Hence AB-interaction between particle size and time, AC-interaction between particle size and solvent type, BC-interaction between time and solvent type and B^2-the square of time are not significant model terms.

From Design expert soft ware model statistics summary, quadratic model was suggested as shown in the table 4.9 below.

Table 4.6 Model statistics summary

Source	Standard Deviation	R squared	Adjusted R squared	Predicated R squared	Press	Remark
Linear	6.08	0.9062	0.8892	0.8608	1207.83	
2FI	6.19	0.9250	0.8853	0.8197	1564.13	
Quadratic	4.56	0.9641	0.9377	0.8784	1054.86	**Suggested**
Cubic	2.93	0.9931	0.9742	0.8699	1128.36	

Considering ANOVA table (4.8) the model terms A, B, C and A^2 were significant model terms where as interaction model terms AB, AC, BC and B2 are not significant model terms. Often we think about removing non significant model terms or factors from a model but in this case removing AB, AC and BC will result in a model that is not hierarchical. The hierarchy principle indicates that if a model contains a high- order term, it should contain all the lower- order terms that compose it. Hierarchy promotes a type of internal consistency in a model, and many statistical model builders rigorously follow the principle (Douglas, 2001).

The final model equation in terms of coded factor was presented by equations 4.1 for representing the variation of percentage oil yield of mango seed kernel with independent factors:

$$\begin{aligned}\text{Yield}\ (\%) = {} & +46.52 - 10.06 * \text{A} + 12.94 * \text{B} + 8.56 * \text{C}[1] - 14.89 * \text{C}[2] - 1.17 * \text{AB} \\ & - 1.85 * \text{AC}[1] + 3.74 * \text{AC}[2] - 0.60 * \text{BC}[1] + 1.32 * \text{BC}[2] + 8.35\text{A}^2 \\ & - 1.91\text{B}^2 \qquad (4.1)\end{aligned}$$

Where: A= Particle size

B= Extraction time

C[1]= Lower limit solvent type

C[2]= Upper limit solvent type

It is evident from equation (4.1) that the coefficients of A and C[2] were negative but that of B and C[1] were positive. Therefore increasing the particle size and using the upper limit solvent type will decrease the percentage oil yield. Whereas increasing the extraction time and using the lower limit solvent type will increase the percentage oil yield of mango seed kernel. The coefficient of A^2 was positive therefore it will show positive quadratic effect on oil yield.

Final Equation in Terms of Actual Factors:

Solvent type: Hexane

$$\text{Yield}\ (\%) = +52.7495 - 35.36967 * \text{Particle size} + 10.81294 * \text{Time} - 0.62318 * \text{Particle size} * \text{Time} + 9.55246 * \text{Particle size}^2 \quad (4.2)$$

Solvent type: Ethanol

$$\text{Yield}\ (\%) = +17.61893 - 29.39582 * \text{Particle size} + 11.77044 * \text{Time} - 0.62318 * \text{Particle size} * \text{Time} + 9.55246 * \text{Particle size}^2 \quad (4.3)$$

Solvent type: Petroleum ether

$$\text{Yield}\ (\%) = +50.8218 - 35.40921 * \text{Particle size} + 10.7510 * \text{Time} - 0.62318 * \text{Paricle size} * \text{Time} + 9.55246 * \text{Particle size}^2 \quad (4.4)$$

Diagnostics

The following figure (4.7) shows the relation between the actual value of the experiment and value predicated by the model equation developed by the design expert soft ware 7.0.0

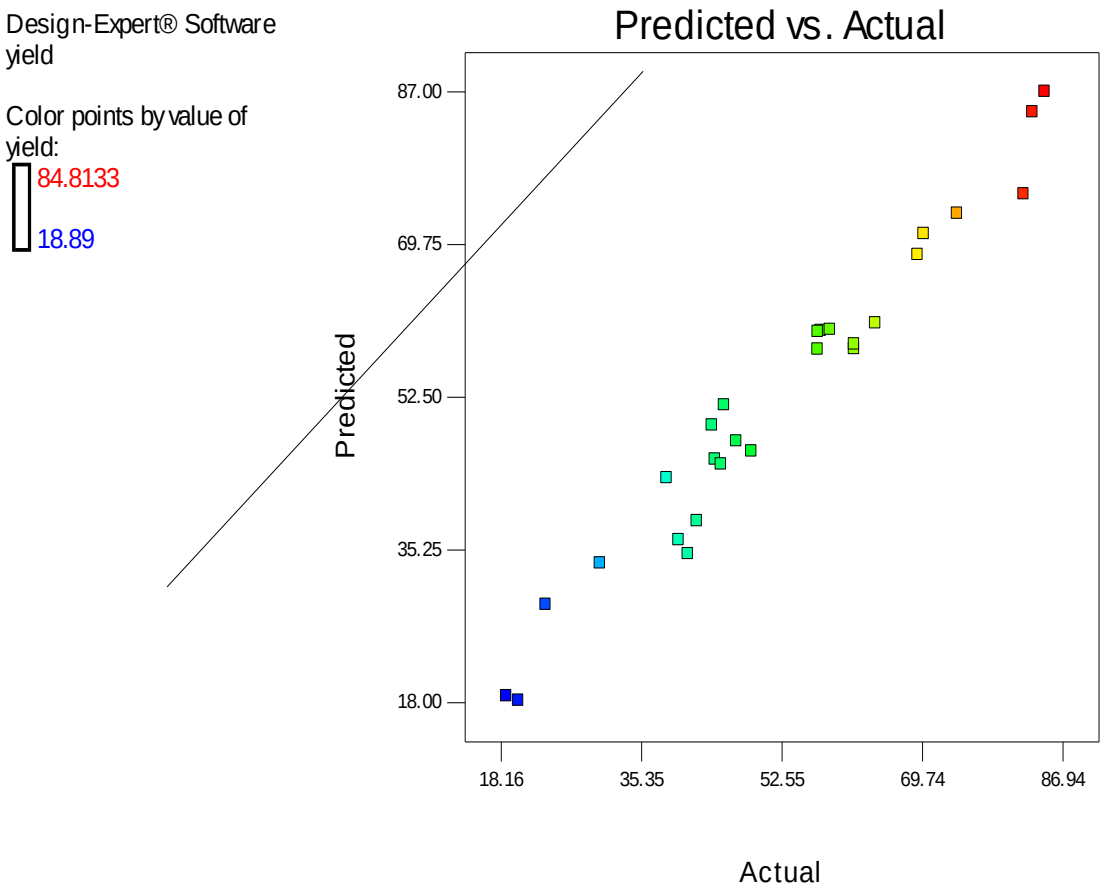

Figure 4.7 Actual value verses predicated value of percentage oil yield

The residual, the difference between the actual value of the experiment and the predicated value (which was calculated using equation (4.2), (4.3), and (4.4) for some of the runs are shown in the table 4.10)

Table 4.7 Difference between the experimental (actual) value and predicated value

Standard order	Actual value	Predicated value	Residual
Solvent type: Hexane			
1	57.41	61.84	-4.43
2	44.44	47.31	-2.87
3	42.22	40.34	1.88
4	82.22	82.99	-0.77
5	58.52	67.68	-9.16
Solvent type: Ethanol			
1	23.70	30.89	-7.19
2	20.37	20.57	-0.2
3	18.89	20.57	-1.68
4	48.89	53.96	-5.07
5	41.11	42.36	1.25
Solvent type: Petroleum ether			
1	61.48	59.77	1.71
2	38.52	45.22	-6.7
3	40.0	38.21	1.79
4	74.07	80.80	-6.73
5	57.02	65.48	-8.46

4.4 Characterization of the extracted oil

Using process parameter that gave a maximum oil yield (particle size range 0.25-0.5mm, extraction time = 6hr and solvent type hexane) oil was extracted and physical and chemical properties were studied. But, for the case of phenolic content test, three different oils were extracted by three solvents under 6 hr and 0.25-0.5mm extraction time and particle size range respectively.

4.4.1 Moisture and volatile matter of oil

The moisture and volatile matter of the oil was determined by oven method. 5 gm of oil was taken and put in oven and the weight was recorded at 1 hour and 2 hours. The result obtained is summarized in the table below.

Time (hr)	0	1	2
Weight (gm)	5.0	4.89	4.89

From equation (GA.2)

W1 is loss in gm in material on drying = 5.0gm – 4.89gm = 0.11gm

W is weight in gm of oil taken for the test = 5.0gm

Substituting the above values in the equation (3.3)

$$\text{Moisture and vilatile matter} = \frac{0.11\text{gm}}{5.0\text{gm}} * 100\%$$

$$= 2.2\%$$

4.4.2 Specific gravity

Density bottle method was used to determine the specific gravity of oil as the detail experimental procedures were stated in section 3.4.1.2.

From equation (GA.3);

A is weight in gm of density bottle with oil at 30^0C = 35.60

B is weight in gm of density bottle at 30^0C = 13.60

C is weight in gm of density bottle with water at 30^0C = 37.9

Substituting the above values in the equation (GA.3)

$$\text{Specific gravity} = \frac{35.60 - 13.60}{37.9 - 13.6}$$

$$= 0.905$$

Hence the density of oil can be determined using:

$$\text{SG} = \frac{\rho_{\text{oil}}}{\rho_{\text{W}}}$$

Where: ρ_{oil} = density of mango seed kernel oil

ρ_W = density of water = 1000 kg/m^3

Therefore density of oil was 905 kg/m^3

4.4.3 Kinematic viscosity

Dynamic viscosity of oil, which was red from vibro viscometer, was 49.1mpa.s at a temperature of 30.2^0C.

Substituting the dynamic viscosity of oil = 49.1mpa.s = 4.91*10^{-4}kg/m.s and density of oil = 905kg/m^3 in equation (GA.4)

$$\text{Kinematic viscosity} = \frac{4.91 * \frac{10^{-4}\text{kg}}{\text{m}}.\text{s}}{\frac{905\text{kg}}{\text{m}^3}}$$

$$= 5.43*10^{-5}\text{m}^2/\text{s}$$

Therefore the kinematic viscosity of oil was 5.4*$10^{-5}$$m^2$/s

4.4.4 pH value of oil

The pH value of oil was determined by pH electrode as measuring experimental procedures were stated in section 3.4.1.4. The pH value of kernel oil was triplicated and the results obtained are summarized in table 4.11 below.

Table 4.8 pH value of mango seed kernel oil

Run	pH value	Mean ± SD
1	6.3	5.9±0.36
2	5.6	
3	5.8	

Therefore the pH value of mango seed kernel oil was from 5.6 to 6.5 which is slightly neutral. In preparation of skin and hair care materials, the preferable pH value is in the range of 3.5 – 6.5 (Mueller et al., 2000). The obtained pH value of kernel oil is in the range to be used in producing cosmetic materials.

4.4.5 Refractive index

Refractometer was used to determine the refractive index of the kernel oil and A.O.C.S official method 921.08 was implemented. A refractive index of 1.45584 at a temperature of 40^0c was obtained. Refractive index indicate the purity of oil. The lower the refractive index is the higher the quality of oil (Anhwange et al., 2010). The result obtained indicated that the oil is of high quality.

4.4.6 Saponification value

Saponification number was determined by using titration. Saponification is a number that expresses in milligram of quantity of potassium hydroxide required to saponify 1 gram of oil/fat. The required solutions were prepared with the required concentration.

- ✓ Preparation of 0.5N alcoholic potassium hydroxide solution:
  - ✓ $\frac{5.6\text{gm}}{0.85} = 6.59\text{gm}$ of 85 percent pure KOH was dissolved in 200ml 80 percent ethanol.
- ✓ Preparation of 0.5N hydrochloric acid solution:
  - ✓ A stock solution of 13.7ml of hydrochloric acid was poured in to 500ml distilled water.

After 2 gm of fat was dissolved in alcoholic KOH and heated gently for 1 hour, it was titrated with HCl to the end point. Similar titration was done for the blank. In both case the value of HCl was recorded.

Saponification value was calculated using equation (GA.5)

B is volume of HCl required for the blank = 37.6ml

S is volume of HCl required for the sample = 24.4ml

N is normality of HCl = 0.5N

W is weight of fat taken for the test = 2 gm

Substituting the above values in equation (GA.5)

$$\mathrm{SV} = \frac{56.1 * (37.6\mathrm{ml} - 24.4\mathrm{ml}) * 0.5\mathrm{N}}{2\mathrm{gm}}$$

SV = 186.53mgKOH/gm

Table 4.9 Saponification value of mango seed kernel oil

Run	Volume of HCl for the blank (ml)	Volume of HCl for the sample (ml)	Massof sample(gm)	SV
1	37.6	24.4	2	186.53
2	37.6	22.9	2	206.17
3	37.6	26.1	2	161.29
			Mean±SD	184.66±22.5

Hence, the saponification value of mango seed kernel oil was 184.66 mgKOH/gm of oil. High saponification value implies greater proportion of fatty acids of low molecular weight. The values obtained for saponification value of mango seed kernel oil was favorably comparable with the saponification value of olive oil (185 - 196) which is a well known vegetable oil in cosmetics industry. High saponification value of the mango kernel oil suggests the use of the oil in production of liquid soap, shampoos and lather shaving creams.

4.4.7 Unsaponifiable matter

Titration method was implemented for the determination of unsaponifiable matter of mango seed kernel oil. As the detail experimental procedure was stated in section 3.4.2.2. The required solutions were prepared with a required concentration.

- ✓ Preparation of 0.02N sodium hydroxide (NaOH) solution:
  - ✓ 0.81gm of 99 percent NaOH was dissolved in 1000ml distilled water.
- ✓ Preparation of alcoholic potassium hydroxide (KOH) solution:
  - ✓ 1.32gm 85 percent pure KOH was dissolved in 1000ml 80 percent ethanol.

From equation (GA.6)

A is weight of the residue

Mass of flask = 102.6440gm

Mass of flask + mass of residue = 102.9042gm

∴ Mass of residue = 102.9042gm – 102.6440gm = 0.2601gm

V is volume of sodium hydroxide solution = 12ml

N is normality of sodium hydroxide solution = 0.02N

Substituting the above values in equation (GA.6)

$$\text{Unsaponifiable matter} = \frac{100(0.2601 - 0.282 * 12 * 0.02)}{5}$$

$$= 3.85\%$$

The result obtained is in agreement with the reported values by Nzikou et al.,(2010), Saiprabha et al.,(2011) and Abdela et al., (2007) who reported 4.35%, 3.45% and 2.78% respectively.

Unsaponifiable matters are substances soluble in oil which fails to form soap when blended with sodium hydroxide. They are insoluble in water but soluble in the solvent used for the determination. It includes lipids of natural origin majority sterols and tocopherols (vitamin E) and higher aliphatic alcohols, pigments, vitamins as well as foreign organic matter non volatile at 100^0C e.g. (mineral oil) (FAO; Maryam et al., 2013)

Presence of high unsaponefiable matter in oil guarantees the use of oils in cosmetics industry since the most widely used vitamin in cosmetics product is vitamine E and majority of unsaponifebility is tocopherols (vitamin E) and sterols. In cosmetics product vitamin E, in addition to its ability to quench free radicals, significantly decreases skin wrinkling and is also excellent moisturizing effect. Hence, the presence of high unsaponifeble matter, 3.85 guarantees the use of mango seed kernel oil in cosmetics industry.

4.4.8 Acid value

Acid value is the measure of total acidity of the lipid involving contributions from all the constituent fatty acids that make up the glyceride molecule (Ekpa and Ekpe, 1995). Titration method was used to determine the acid value. The required solutions were prepared with the required concentration as follows.

- Preparation of 80 percent ethyl alcohol: 19.6ml distilled water was added in to 80.4ml 99.5 percent absolute ethanol.
- Preparation of 0.5N sodium hydroxide solution: 10.1gm of 99 percent NaOH was disloved in 500ml distilled water.

From equation (GA.7)

V is volume of NaOH used by the sample during titration = 0.25ml

N is normality of NaOH = 0.5N

W is weight of oil taken for test = 3.005gm

Substituting the above values in equation (GA.7)

$$\mathrm{AV} = \frac{\frac{56.1\mathrm{gm}}{\mathrm{mol}} * 0.25\mathrm{ml} * 0.5\mathrm{N}}{3.005\mathrm{gm}}$$

AV = 2.33mgKOH/g of oil

$$\text{Percent free fatty acid} = \frac{\mathrm{AV}}{2} = \frac{2.33}{1.99} = 1.17$$

Triplicate result obtained is summarized in the table 4.13

Table 4.10 Acid value for mango seed kernel oil.

Run	Titration volume	AV	%FFA
1	0.25	2.33	1.17
2	0.28	2.61	1.30
3	0.23	2.15	1.08
Mean±SD		2.38±0.33	1.18±0.11

From table 4.13 the average acid value of mango seed kernel oil is 2.38±0.33 which is relatively smaller. The low acidity of an oil is an indication of oil which is free from hydrolytic rancidity and enables the direct use of such oil without further neutralization (Arogba, 1999). Therefore the result obtained indicated that mango seed kernel oil can be used directly without further neutralization.

The low free fatty acids content (1.18) was indicative of low enzymatic hydrolysis. This can be an advantageous that mango seed kernel oil cannot develop flavor during storage.

4.4.9 Iodine value

The iodine value (IV) is the amount of iodine (in grams) necessary to saturate 100 g of oil sample. The iodine value is used to determine the unsaturation of oils and in assessing the stability of oil in industrial applications (Xu et al., 2007). The lower the iodine value of oil, which reflects its characteristics such as higher resistance to oxidation, the longer shelf life and higher quality. Whereas the higher the iodine value of oil, the lower the quality.

Testing of Iodine value of mango seed kernel fat has been conducted at JIJE Analytical testing service laboratory plc and it was found to be 40.44 wij's gm/100gm of oil. The result indicated that mango kernel oil has low iodine value, which indicates high resistance to oxidation and longer shelf life. The oil can be classified as a non-drying oils since its iodine value is lower than 100. Certainly, the oil can also be used extensively as lubricants and hydraulic brake fluids.

4.4.10 Peroxide value

Frequently used tests employed to predict the quality of seed oils is the determination of peroxide value and iodine value.

Peroxide value is one of the most widely used testing for oxidative rancidity in oils. It is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. Generally, the peroxide value should be less than 10 mg/g oil in the fresh oils.

Peroxide value of mango seed kernel fat has been conducted at JIJE Analytical testing service laboratory plc and found to be 2.92meq peroxide oxygen/kg.

Ojeh (1981) reported that oils with high peroxide values are unstable and become easily rancid.. So mango seed oil had a high quality due to the low level of peroxide value.

4.4.11 Total phenolic content

Folin ciocaletea method was used to determine total phenolic content of mango seed kernel oil as the experimental procedure were stated in section 3.4.2.6.

The required solutions with the required concentration were prepared as follows.

- ✓ Preparation of six known concentration of galic acid:
  - ✓ 1.5 g of galic acid was dissolved in 300 ml of distilled water to obtain 5 g/lit of the final stock. A standard concentration of 50 mg/ml, 100 mg/ml, 200 mg/ml, 250 mg/ml, 400 mg/ml, and 500 mg/ml was created by dissolving 10 ml, 20 ml, 40 ml, 50 ml, 80 ml and 100 ml from the stock in 1 ml of distilled water.
- ✓ Preparation of 7.5% sodium carbonate solution:
  - ✓ 7.5gm of anhydrous sodium carbonate was dissolved in 92.5ml distilled water and brought to boil (saturated). After it was cooled few crystals of Na_2CO_3 added and it was left for 24 hour at room temperature. And then it was filtered through whatman No 1 filter paper.
- ✓ 10% folin ciocaletea reagent preparation: 8ml folin ciocaletea was dissolved in 72 ml distilled water.

After 50, 100, 200, 250, 400, and 500mg/ml concentration galic acid solutions were prepared, 0.4ml from each concentration and from oil samples were taken and 2ml 10% folin ciocaltea reagent, 1.6ml 7.5% Na_2CO_3 and 7 ml distilled water were added. The absorbances were red in duplicate using UV visible spectrophotometer after they were left for 30 minute at room temperature and the following results were recorded.

Table 4.11 Absorbation for known concentration of galic acid

Concentration (mg/ml)	Absorbance	Mean absorbance (mean)
50	1. 0.2628	0.2623
	2. 0.2616	
100	1. 0.5516	0.5367
	2. 0.5218	
200	1. 0.9474	0.9168
	2. 0.8862	
250	1. 0.9962	1.0326
	2. 1.0690	
400	1. 1.4787	1.4690
	2. 1.4592	
500	1. 1.6833	1.6415
	2. 1.5997	

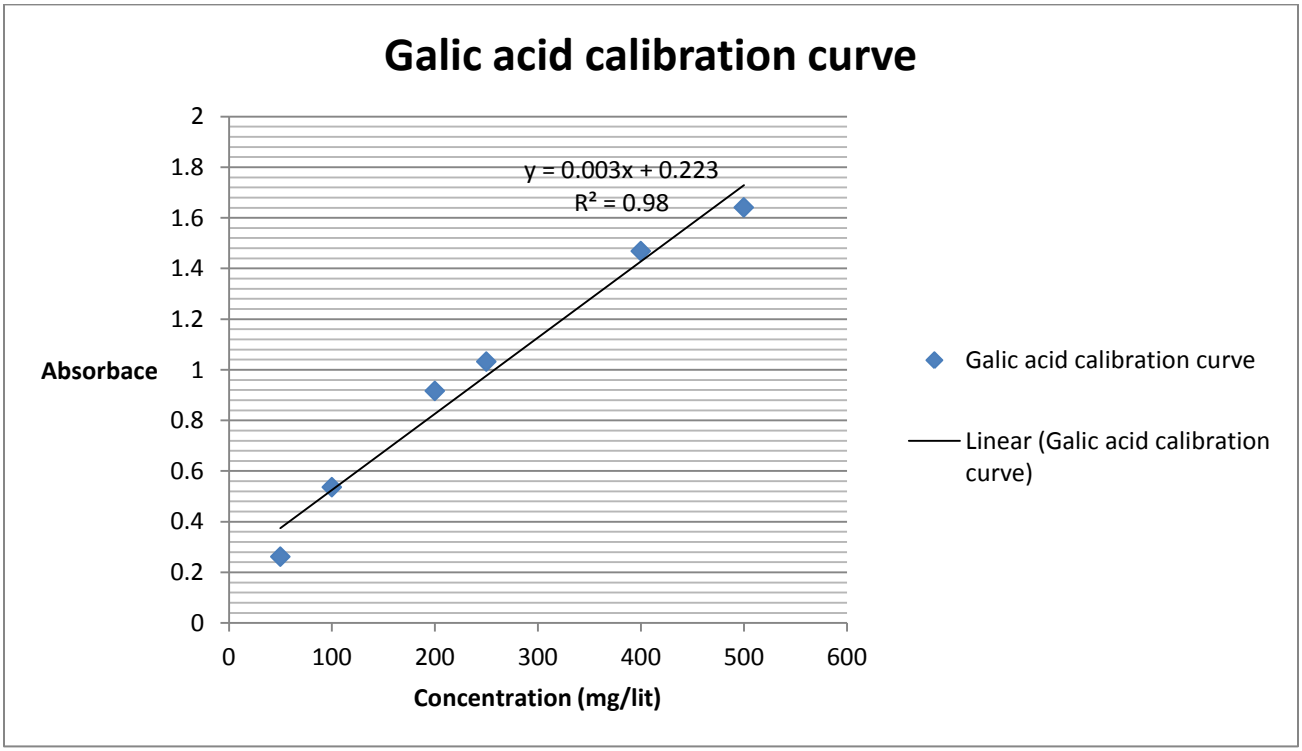

Figure 4.8 Galic acid calibration curve

The absorbance of mango seed kernel oil extracted with the three solvent (hexane, ethanol and petroleum ether) were red and the following result were obtained. The corresponding concentration were calculated using the $X = -74.333 + 333.333Y$; $R^2 = 0.98$

Table 4.12 Absorbance of mango seed kernel

Oil	Absorbance	Mean±SD	Concentration (mg/ml)
Oil extracted with hexane	1. 0.7390	0.4489±0.37	75.30
	2. 0.0357		
	3. 0.5719		
Oil extracted with ethanol	1. 0.4328	0.5374±0.21	104.80
	2. 0.7761		
	3. 0.4032		
Oil extracted with petroleum	1. 0.4125	0.4391±0.05	72.03
	2. 0.4963		
	3. 0.4084		

Total phenolic content was calculated using equation (3.9) and a total phenolic content of 83.2 mg/g, 115.8 mg/g and 79.6 mg/g obtained for hexane, ethanol and petroleum ether extracts respectively were obtained as shown in table (4.15). A total phenolic content of 98.7mg/g was reported by Kittiphoom and Sutasinee (2013) and also 118.1mg/g and 117mg/g was reported by Saranyu and Rakrudee (2011) and Nzikou et al. (2010) respectively.

The oil extracted with ethanol resulted in high phenolic content followed by hexane and petroleum ether. Ethanol resulted high phenolic content, this may be due to the fact that ethanol is polar organic solvent and phenolic compoundes are very polar (Tsimidou et al., 1992) so ethanol can dissolve more phenolic compound. The potential use of phenolic compounds for the development of new skin care cosmetics has been emphasized. Phenolic compounds can be used as skin whitening, sunscreen and anti-wrinkle agents (González et al., 2008). In addition, phenolic compounds are the main component responsible for antioxidant activity. This is mainly due to their redox property which can play an important role in absorbing and neutralizing free radicals

There is a positive correlation between TPC and free radical scavenging abilitity (Gorinstein et al. (2003). Gorinstein et al. (2003) compared, the contents of the main biochemical compounds and the antioxidant activity of some Spanish olive oils and found that the correlation of TPC and the radical scavenging capacity was very high (R= 0.9197-0.9958). So the presence of high phenolic content in mango seed kernel implies that high free radical scavenging activity.

The result obtained indicated that mango seed kernel oil is a good source of phenolic compounds. Therefore, the oil can successfully be used in cosmetics formulation in cosmetics industry for the production of lotion, shampoo and soap or other body and hair caring cosmetics material.

4.5 Comparison of oil physicochemical properties

The comparisons of oil physicochemical property against both commercially availablea and the standared specification are given in the table 4.13

Oil properties	Measured value	Commercial (JEEN international)	Indian standard (FSSAI)
Moisture (%)	2.2	-	
Specific gravity	0.905	0.910	0.908
Refractive index	1.45584	1.4532	1.4550 - 1.4604
pH value	6.07	-	-
Viscosity	$5.4*10^{-5}$	Semisolid @room Tem	Semisolid@room Tem
Saponification value (mgKOH/g)	184.66±22.5	185 - 195	185 - 198
Unsaponifeble matter (%)	3.85	1	1.5
Acidvalue (mgKOH/g)	2.38±0.33	0.5	1.5
Iodine value (wijs g/100g)	40.44	55-65	32 -57
Peroxide value (meq peroxide oxygen/kg)	2.92	Max 5.0	-
Phenoliccontent (mg GAE/g oil)			
Hexane extract	83.2		
Ethanol extract	115.8		
Petroleum extract	79.6		

The physicochemical properties of the oil were within the standards (FSSAI) and commercial available specification except the acid value and unsaponifiable matter which were slightly higher. The phenolic content of the oil was not compared due to the lack of data under this specific property. However, it can be said that the higher unsaponifiable matter in Ethiopian mango (local variaty) seed oil will give higher opportunity to be used in cosmetics industry since high unsaponifeble matter in the oil guarantees the use of oil in cosmetics industry (Nzikou et al., 2010; Saiprabha et al., 2011).

Chapter five

Conclusion and recommendation

5.1 Conclusion

In this research oil was extracted from mango seed kernel using soxhlet extractor. Particle size, solvent type and extraction time were the considered parameters for optimization investigation. Under this investigation particle size range; 0.25-0.5mm, 0.5-1.5mm and 1.5-3mm, solvent type; hexane, ethanol and petroleum ether and extraction time 2 hr, 4 hr and 6 hr were considered.

From the experimentation it was found that maximum oil yield of 84.81% was obtained at particle size range of 0.25-0.5mm, solvent type hexane and extraction time of 6 hour followed by a yield of 83.33% at particle size range 0.25-0.5mm, solvent type petroleum ether and 6 hour extraction time. A minimum oil yield of 18.87% was obtained at particle size range of 0.5-1.5mm, solvent type ethanol and 2 hour extraction time followed by a yield of 20.37% at particle size range of 1.5-3mm, solvent type ethanol and 2 hour extraction time. According to the result obtained the yield of petroleum ether is almost equal to that of hexane. Since the boiling point range of petroleum ether (40^0C – 60^0C) is lower than that of hexane (65^0C – 69^0C) to avoid thermal degradation of bioactive components it is preferable to use petroleum ether than hexane.

From design expert soft ware the analysis of ANOVA P value < 0.0001 for particle size, extraction time and solvent type indicated that operating parameters have significant effect on oil yield.

After the optimum operating condition for maximum oil yield was determined, using the optimum operating condition the oil was extracted and the physicochemical properties of the oil like phenolic content, unsaponifeble matter, acid value, peroxide value, saponification value, iodine value, pH, moisture and volatile matter, refractive index and specific gravity were determined for characterization and quality analysis. The results were compared with commercial available mango kernel oil specifications and with Indian standard and the values were within the standards except the acid value which is slightly higher.

From phenolic content test, it was found that the oil extracted by ethanol as a solvent had a higher value than that of hexane and petroleum ether as a solvent. Even if the oil yield obtained by ethanol lower by 3.34% from that of hexane, due to its high yield of phenolic content, the

greenness, low cost and availability of ethanol, ethanol is suggested as best solvent for the extraction of oil from mango seed kernel for cosmetics application.

Generally due to the presence of high unsaponifeble matter (3.85%) and phenolic content, the oil extracted from mango seed kernel guarantees the use of oil for cosmetics industry. High saponification value of oil suggests the use of oil in production of liquid soap, shampoos and lather shaving creams.

5.2 Recommendations

Even if mango kernel oil has been used worldwide in formulation of cosmetics product, in Ethiopia peoples ignorantly throw away the mango seeds after eating the fruit part and it becomes a source of pollution. The present study has enables as the use of mango kernel oil in cosmetics formulation so I recommend further studies to be performed if necessary on the performance of the oil and to be used in cosmetics industry for the production of hair and body butters and production of the oil in large scale and exporting and can be a source of income for the country.

Here, the fatty acid composition of has not been studied. This is due to the lack of standard in GC analysis for the test so further study should be done in fatty acid composition test of the oil.

Moreover, the detail techno economic feasibility study for both large scale production and small scale manufacturing of mango seed kernel oil demands future study.

Additionally, I recommend the effect of solid to solvent ratio and effect of temperature on oil yield to be performed.

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Annexes

Annex A: Table A1 Fatty acid composition of mango seed oil

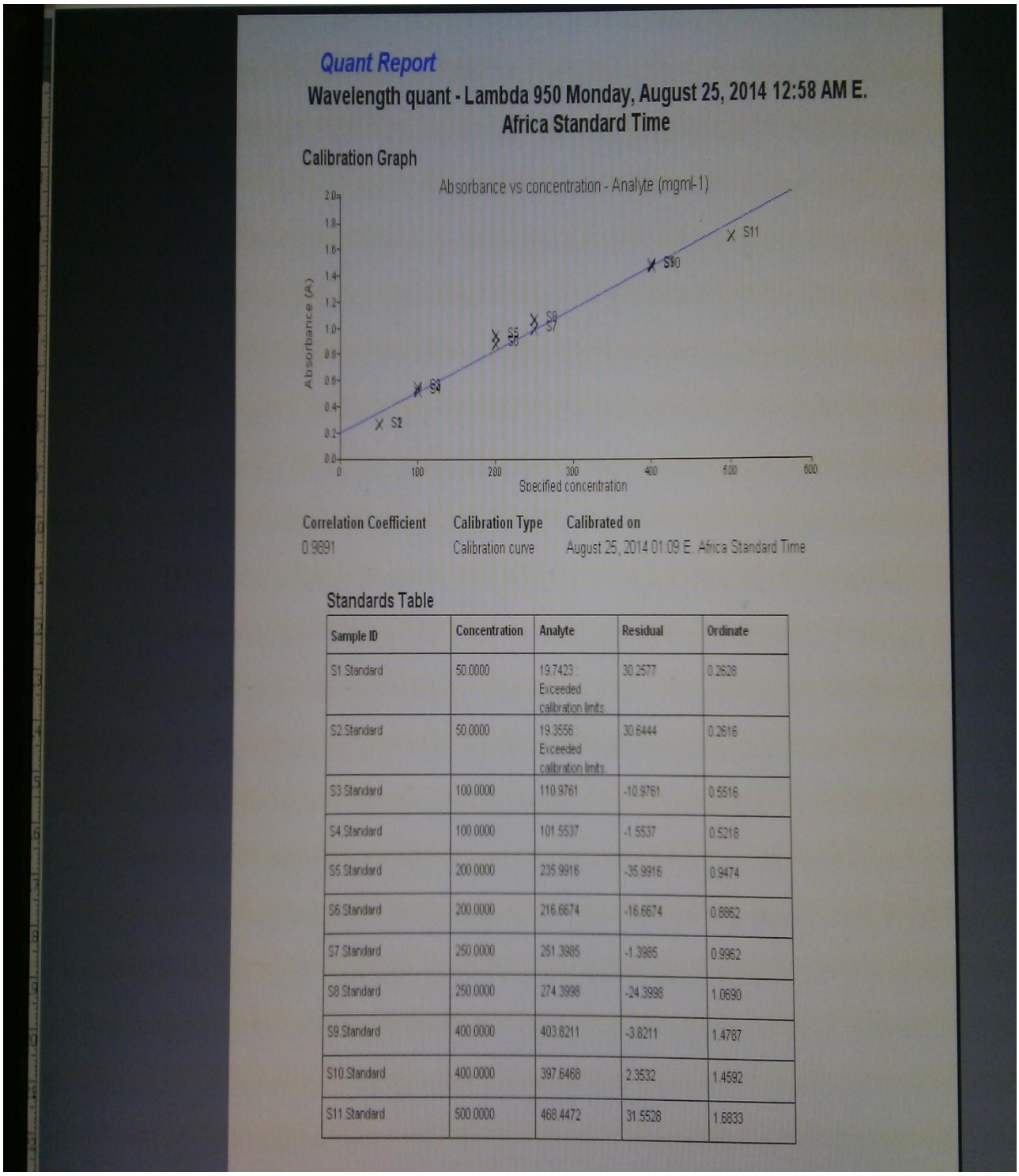
Fatty acid	Formula	Structure	Percent (%)
Palmitic acid	$C_{16}H_{32}O_2$	16:0	6.48
Srearic acid	$C_{18}H_{36}O_2$	18:0	37.94
Olic acid	$C_{18}H_{34}O_2$	18:1	45.76
Linoleic acid	$C_{18}H_{32}O_2$	18:2	7.45
Linolenic acid	$C_{18}H_{30}O_2$	18:3	2.37
Saturated			44.42
Unsaturated			55.58

(Source: Nzikou et al., 2010)

Table A2 Fatty acid composition of different vegetable oils

	Fatty acid composition (wt %)															
Vegetable oil	12:0	14:0	14:1	16:0	16:1	18:0	20:0	20:1	22:0	24:0	18:1	22:1	18:2	18:3	18:4	6:0, 8:0, 10:0 and others
Cottonseed	-	0	-	28	-	1	0	-	0	0	13	0	58	0	-	-
Tobacco	-	0.09	-	10.96	0.2	3.34	-	-	-	-	14.54	-	69.49	0.69	-	0.69
Rapeseed	-	0	-	3	-	1	0	-	0	0	64	0	22	8	-	-
Safflower	-	0	-	9	-	2	0	-	0	0	12	0	78	0	-	-
Sunflower	-	0	-	6	-	3	0	-	0	0	17	0	74	0	-	-
Sesame	-	0	-	13	-	4	0	-	0	0	53	0	30	0	-	-
Lindseed	-	0	-	5	-	3	0	-	0	0	20	0	18	55	-	-
Palm tree	-		-	35	-	7	-	-	-	-	44	-	14	-	-	-
Corn	-	0	-	12	-	2	Tr	-	0	0	25	0	6	Tr	-	-
Tallow	-	-	-	23.3	0.1	19.3	-	-	-	-	42.4	-	2.9	0.9	2.9	-
Soya bean	-	-	-	14	-	4	-	-	-	-	24	-	52	-	6	-
Peanut	-	0	-	11	-	2	1	-	2	1	48	0	32	1	-	-
Coconut	48.8	19.9	-	7.8	0.1	3.0	-	-	-		4.4	-	0.8	0	65.7	8,9, 6,2
Yellow grease		0.70	0.00	14.26	1.43	8.23	0.33	0.48	-	-	43.34	-	26.25	2.51	0.47	-

Annex B: Galic acid calibration curve output from uv- visible spectrophetometry



Quant Report

Wavelength quant - Lambda 950 Monday, August 25, 2014 12:58 AM E. Africa Standard Time

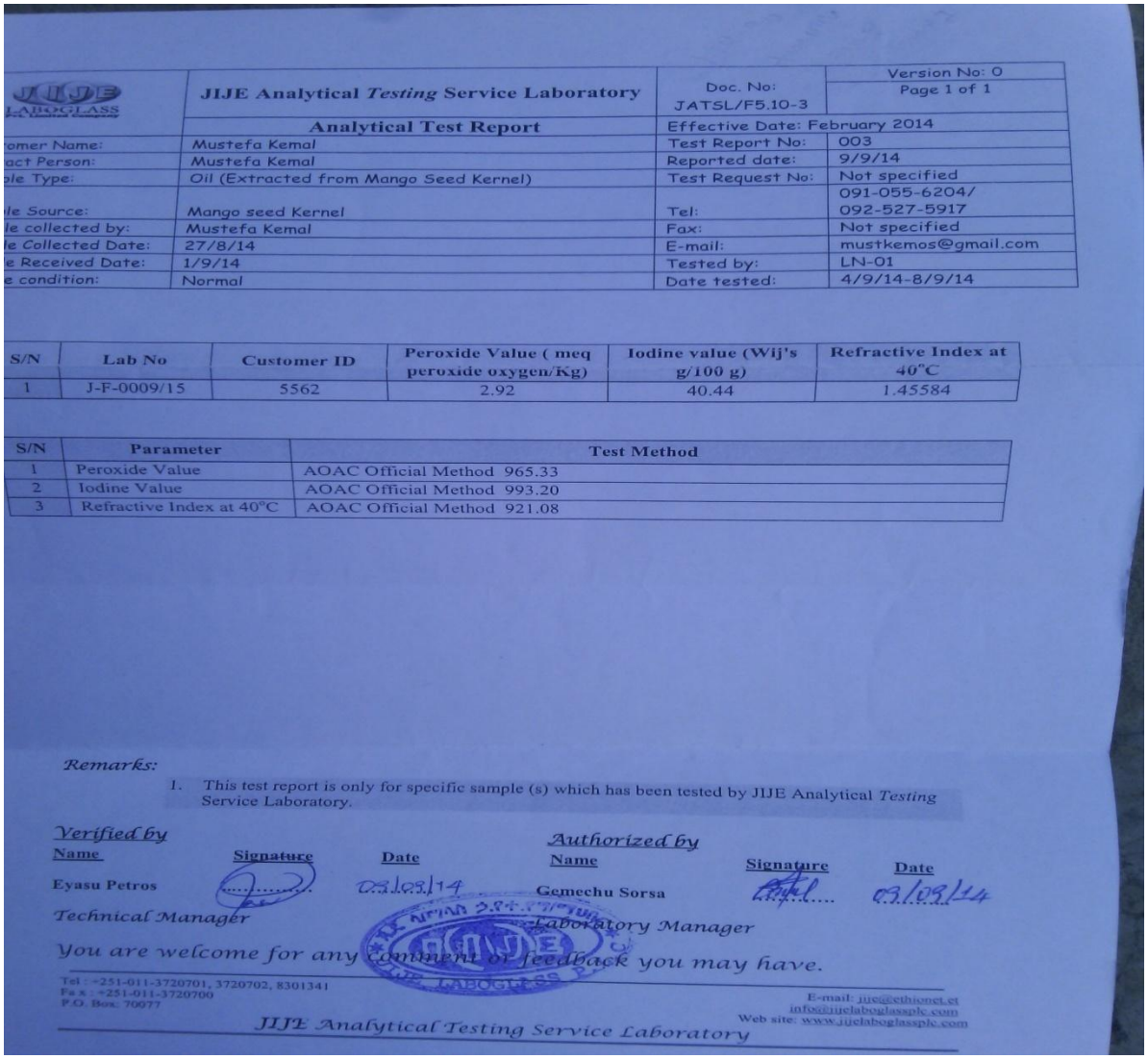
Calibration Graph

Correlation Coefficient	Calibration Type	Calibrated on
0.9891	Calibration curve	August 25, 2014 01:09 E. Africa Standard Time

Standards Table

Sample ID	Concentration	Analyte	Residual	Ordinate
S1 Standard	50.0000	19.7423 Exceeded calibration limits	30.2577	0.2628
S2 Standard	50.0000	19.3556 Exceeded calibration limits	30.6444	0.2616
S3 Standard	100.0000	110.9761	-10.9761	0.5516
S4 Standard	100.0000	101.5537	-1.5537	0.5218
S5 Standard	200.0000	235.9916	-35.9916	0.9474
S6 Standard	200.0000	216.6674	-16.6674	0.8862
S7 Standard	250.0000	251.3985	-1.3985	0.9962
S8 Standard	250.0000	274.3998	-24.3998	1.0690
S9 Standard	400.0000	403.8211	-3.8211	1.4787
S10 Standard	400.0000	397.6468	2.3532	1.4592
S11 Standard	500.0000	468.4472	31.5528	1.6833

Annex C: Iodine, peroxide and refractive index value for mango kernel oil



JIJE LABOGLASS	JIJE Analytical *Testing* Service Laboratory	Doc. No: JATSL/F5.10-3	Version No: 0 Page 1 of 1
	Analytical Test Report	Effective Date: February 2014	
omer Name:	Mustefa Kemal	Test Report No:	003
act Person:	Mustefa Kemal	Reported date:	9/9/14
ple Type:	Oil (Extracted from Mango Seed Kernel)	Test Request No:	Not specified
le Source:	Mango seed Kernel	Tel:	091-055-6204/ 092-527-5917
le collected by:	Mustefa Kemal	Fax:	Not specified
le Collected Date:	27/8/14	E-mail:	mustkemos@gmail.com
e Received Date:	1/9/14	Tested by:	LN-01
e condition:	Normal	Date tested:	4/9/14-8/9/14

S/N	Lab No	Customer ID	Peroxide Value (meq peroxide oxygen/Kg)	Iodine value (Wij's g/100 g)	Refractive Index at 40°C
1	J-F-0009/15	5562	2.92	40.44	1.45584

S/N	Parameter	Test Method
1	Peroxide Value	AOAC Official Method 965.33
2	Iodine Value	AOAC Official Method 993.20
3	Refractive Index at 40°C	AOAC Official Method 921.08

Remarks:

1. This test report is only for specific sample (s) which has been tested by JIJE Analytical *Testing* Service Laboratory.

Verified by

Name: Eyasu Petros — Signature: ..... — Date: 03/09/14

Technical Manager

Authorized by

Name: Gemechu Sorsa — Signature: ..... — Date: 03/09/14

Laboratory Manager

You are welcome for any comment or feedback you may have.

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JIJE Analytical Testing Service Laboratory

Annex D: Laboratory setups

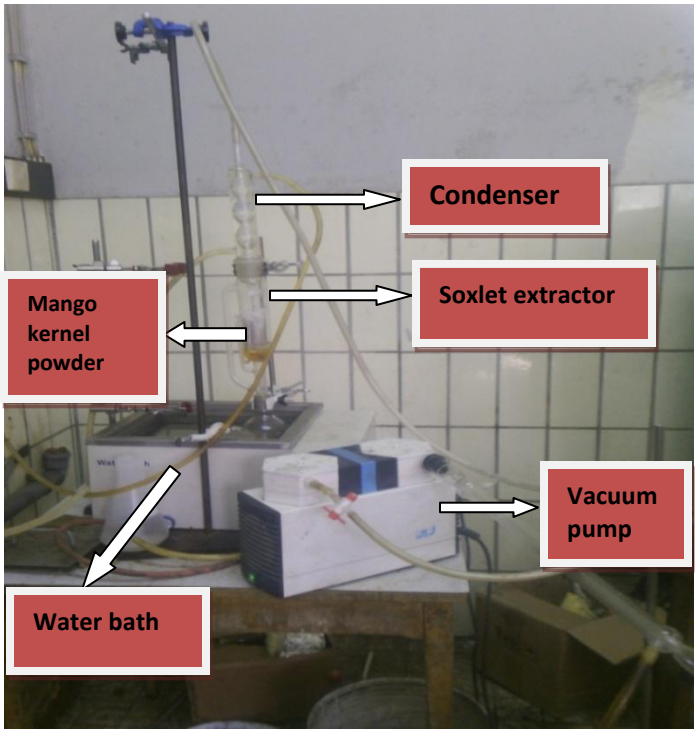


Figure 3.1 Laboratory setup for solvent type hexane and ethanol

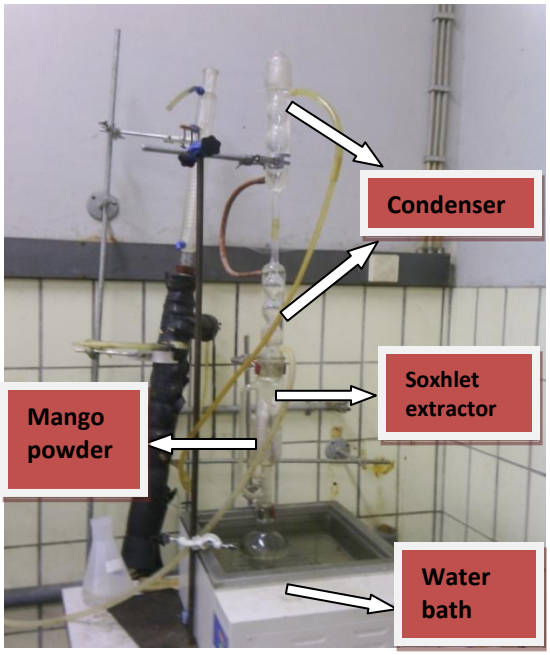


Figure 3.2 Setup when petroleum ether solvent is used

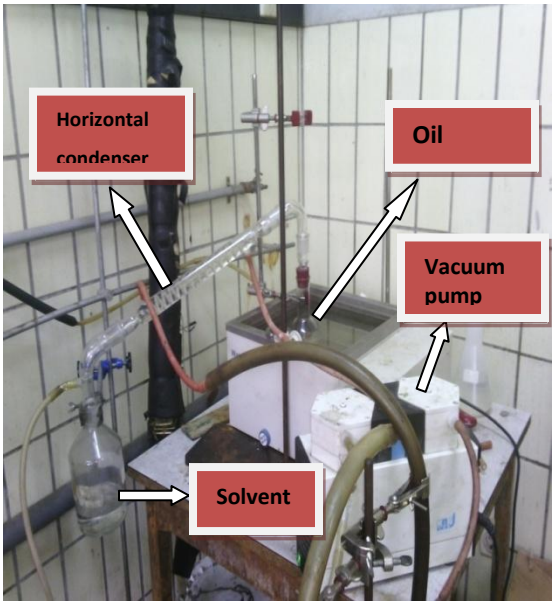


Figure 3.3 Setup for separation process

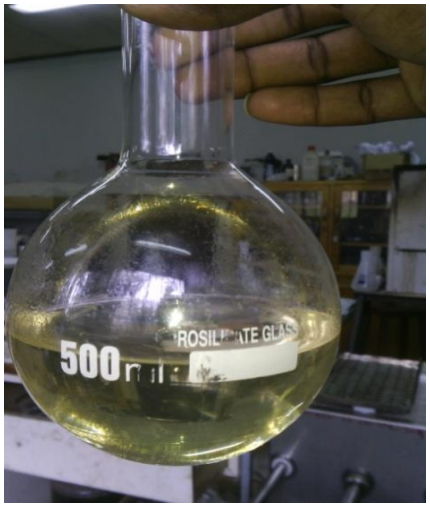
Annex E: Laboratory sample photo

Dried mango kernel

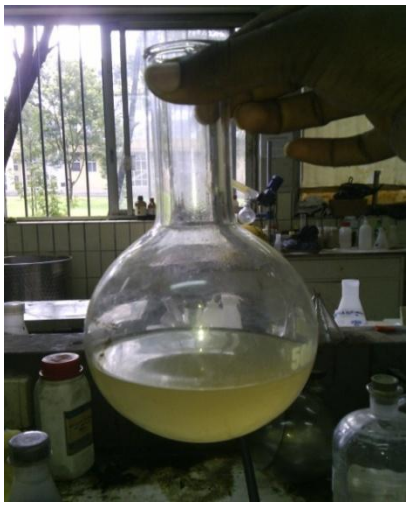
Sieve analysis of mango kernel powder

Three different particle sizes of mango kernel powder

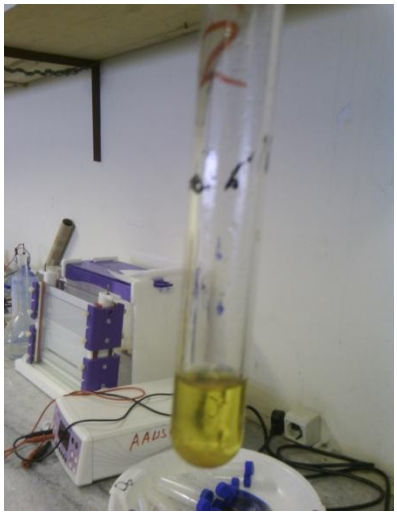
After extraction, oil plus ethanol solvent



After extraction, oil plus hexane solvent



After extraction, oil plus petroleum ether solvent

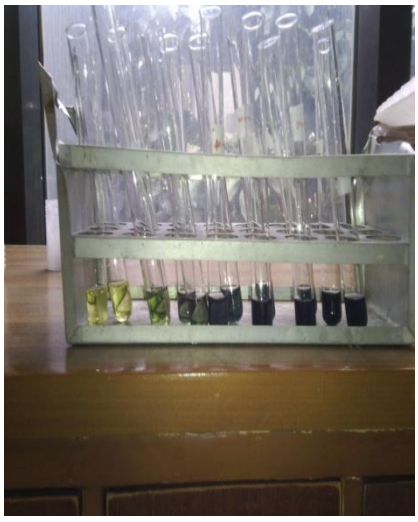


Oil obtained using ethanol solvent

oil obtained using hexane solvent

Oil obtained using petroleum ether

solidified mango kernel oil



Phenolic content determinations

Annex F: full factorial three level and three replica experimental design for three factors

Experiment number	Particle size (mm)	Extraction time (hr)	Solvent type
1	0.25-0.5	2	Ethanol
2	0.25-0.5	4	Hexane
3	0.5-1.5	4	Hexane
4	1.5-3	6	Hexane
5	1.5-3	6	Ethanol
6	0.25-0.5	2	Ethanol
7	0.25-0.5	6	petroleum ether
8	1.5-3	4	Ethanol
9	1.5-3	6	petroleum ether
10	0.25-0.5	2	petroleum ether
11	0.25-0.5	6	petroleum ether
12	0.25-0.5	2	petroleum ether
13	0.25-0.5	2	Hexane
14	0.5-1.5	4	Hexane
15	0.5-1.5	4	Ethanol
16	1.5-3	2	Ethanol
17	0.25-0.5	6	Hexane
18	1.5-3	6	Hexane
19	0.5-1.5	6	petroleum ether
20	0.25-0.5	6	Hexane
21	0.25-0.5	6	Ethanol
22	0.5-1.5	2	Hexane
23	0.5-1.5	4	Ethanol
24	0.5-1.5	6	Ethanol
25	0.5-1.5	2	Ethanol
26	1.5-3	4	petroleum ether
27	1.5-3	2	Ethanol

28	0.5-1.5	4	Ethanol
29	0.25-0.5	4	petroleum ether
30	1.5-3	4	Ethanol
31	1.5-3	4	Ethanol
32	0.25-0.5	6	Hexane
33	0.25-0.5	4	petroleum ether
34	0.5-1.5	2	petroleum ether
35	0.5-1.5	2	Ethanol
36	0.5-1.5	2	petroleum ether
37	0.5-1.5	6	Ethanol
38	1.5-3	6	Ethanol
39	1.5-3	6	petroleum ether
40	0.25-0.5	6	Ethanol
41	0.25-0.5	4	Hexane
42	0.5-1.5	4	petroleum ether
43	0.5-1.5	6	Ethanol
44	1.5-3	2	Hexane
45	0.5-1.5	6	Hexane
46	1.5-3	6	Ethanol
47	1.5-3	4	petroleum ether
48	1.5-3	6	petroleum ether
49	0.5-1.5	4	Hexane
50	0.5-1.5	6	petroleum ether
51	1.5-3	2	petroleum ether
52	0.5-1.5	6	Hexane
53	0.5-1.5	2	petroleum ether
54	1.5-3	4	Hexane
55	0.25-0.5	4	Ethanol
56	1.5-3	4	Hexane
57	0.25-0.5	4	petroleum ether

58	0.25-0.5	4	Hexane
59	0.25-0.5	6	petroleum ether
60	0.5-1.5	2	Hexane
61	1.5-3	2	Hexane
62	1.5-3	4	petroleum ether
63	1.5-3	2	petroleum ether
64	1.5-3	2	Hexane
65	0.25-0.5	4	Ethanol
66	0.25-0.5	4	Ethanol
67	0.25-0.5	2	Hexane
68	0.5-1.5	6	petroleum ether
69	0.25-0.5	2	Hexane
70	0.5-1.5	2	Hexane
71	1.5-3	6	Hexane
72	1.5-3	4	Hexane
73	0.25-0.5	6	Ethanol
74	1.5-3	2	Ethanol
75	0.5-1.5	6	Hexane
76	1.5-3	2	petroleum ether
77	0.25-0.5	2	Ethanol
78	0.5-1.5	4	petroleum ether
79	0.5-1.5	2	Ethanol
80	0.25-0.5	2	petroleum ether
81	0.5-1.5	4	petroleum ether

Annex G: Formulas and Equations used for characterization of the oil

1. Moisture content (%)of the kernel $= \frac{w1-w2}{w1} 100\%$ (AG. 1)

   Where: w1 = Original weight of the sample before drying

   w2 = Weight of the sample after drying

2. Moisture and volatile matter of oil $= \frac{W1}{W} * 100$ (AG. 2)

   Where: w1 = loss in gm of the material on drying

   w = weight in gm of oil taken for the test

3. Specific gravity at 30℃ $= \frac{A-B}{C-B}$ (AG. 3)

   Where: A= weight in gm of density bottle with oil at 30^0C

   B= weight in gm of density bottle at 30^0C

   C= weight in gm of density bottle with water at 30^0C

4. $\nu = \frac{\mu}{\rho}$ (AG. 4)

   Where: μ = dynamic viscosity

   ρ = density of oil

5. Saponification value $= \frac{56.1(B-S)N}{W}$ (AG. 5)

   Where: B= volume in ml of standard hydrochloric acid required for the blank

   S= volume in ml of standard hydrochloric acid required for the sample

   N= normality of hydrochloric acid

   W= weight in gm of the oil/fat taken for the test.

6. Unsaponifiable matter $= \frac{100(A-B)}{W}$ (AG. 6)

   $$B = 0.282VN$$

   Where: A= Weight in gm of the residue

   B= Weight in gm of free fatty acids in the extract as oleic acid

   N= Normality of standard sodium hydroxide solution

   V= volume in ml of standard sodium hydroxide solution

   W= weight in gm of the sample

7. Acid value $= \frac{56.1VN}{W}$ (AG. 7)

Where: V= volume in ml of standared sodium hydroxide solution.

N= Normality of sodium hydroxide solution.

W= weight in gm of sample.

8. $\text{Percent free fatty acid (as oleic acid)} = \frac{\text{AV}}{1.99}$ (AG. 8)

   Where: AV= acid value

9. Total phenolic content (TPC) experessed as milligram of galic acid equivalent per gram of sample is calculated by:

$$\text{TPC} = \frac{\text{CV}}{\text{M}} \quad \text{(AG. 9)}$$

Where: C = concentration of the oil sample in galic acid equivalent

V = volume of oil sample

M = mass of the sample

Annex H: Commercially available mango kernel oil specification

JEEN INTERNATIONAL CORPORATION

24 Madison Road, Fairfield, New Jersey 07004, USA
Tel: 800-771-JEEN (5336), Tel: 973-439-1401, Fax: 973-439-1402,
email: info@jeen.com , Website: www.jeen.com

MANGO KERNAL OIL
INCI: MANGIFERA INDICA (MANGO) SEED OIL

Description
Mango Kernal Oil is composed of refined, fractionated and deodorized vegetable oil taken from the Mango fruit (Mangifera indica). Mango Kernal Oil is semi-solid @ room temperatures.
(INCI: Mango (Magnifera indica) seed oil, EINECS number: 270-311-6, CAS Number: 68424-60-2)
Shelf Life = 2 Years

SPECIFICATION		Corresponding Method of Analysis
Color:	Max 5.0 red	Lovibond 5 ¼"
Acid Value:	Max 0.5	IUPAC 2.201
Peroxide Value (meq/kg)	Max 5.0	AOCS Cd 8b-90
Iodine Value:	55 - 65	IUPAC 2.205
Saponification Value:	185 - 195	

TYPICAL VALUES		
Consistency @ 20°C):	Semi-Solid	Visual
Unsaponifiables (%):	1	AOCS Ca 6a-40
Hydroxyl Value:	9	AOCS Cd 13-60
Density (g/cm^3, @25°C):	0.91	IUPAC 2.101
Melting Point (°C):	15	AOCS Cc 3-25
Cloud Point (°C):	14	AOCS Cc 9a-47
Pour Point (°C):	14	AOCS Cc 9a-47
Refractive Index @ 60°C):	1.4532	
Oxidative Stability (hours @ 110°C):	18	Rancimat

Fatty Acid Composition (%): IUPAC 2.302

C16:0	8	C18:3	1
C18:0	27	C20:0	2
C18:1	52	C22:0	0.5
C18:2	8	others:	1.5

Additives
Citric Acid (%): 0.001

Packaging:
Drum
Net Wt: 408 Lbs.

Mango Kernal Oil is especially prepared for cosmetic and pharmaceutical applications. In the cosmetic industry, Mango Kernal Oil finds application in skin care preparations, creams, lotions and sticks.

Declaration

I declare that the thesis for the M.Sc. degree at the university of Addis Ababa, hereby submitted by me titled “optimization of process parameters for the extraction of oil from mango (Mangifera indica) seed kernel for cosmetics application”, is my original work and has not previously been submitted for a degree at this or any other university, and that all reference materials contained therein have been duly acknowledge.

Mustefa Kemal Osman _____ _____

Name **Signature** **Date**